## Technetium- and Rhenium-Bis(heteroaryl) Complexes, and Methods of Use Thereof

### Related Applications

This application is a continuation-in-part of United States Application serial number 10/386,403, filed March 11, 2003; which claims the benefit of priority to United States Provisional Patent Application serial number 60/363,142, filed March 11, 2002. This application also claims the benefit of priority to United States Provisional Patent Application serial number 60/543,986, filed February 12, 2004; and United States Provisional Patent Application serial number 60/566,635, filed April 29, 2004. The contents of all are hereby incorporated by reference.

### Background of the Invention

Radiopharmaceuticals may be used as diagnostic or therapeutic agents by virtue of the physical properties of their constituent radionuclides. Thus, their utility is not based on any pharmacologic action per se. Most clinically used drugs of this class are diagnostic agents incorporating a gamma-emitting nuclide which, because of physical, metabolic or biochemical properties of its coordinated ligands, localizes in a specific organ after intravenous injection. The resultant images can reflect organ structure or function. These images are obtained by means of a gamma camera that detects the distribution of ionizing radiation emitted by the radioactive molecules.

In radioimaging, the radiolabel is a gamma-radiation emitting radionuclide and the radiotracer is located using a gamma-radiation detecting camera (this process is often referred to as gamma scintigraphy). The imaged site is detectable because the radiotracer is chosen either to localize at a pathological site (termed positive contrast) or, alternatively, the radiotracer is chosen specifically not to localize at such pathological sites (termed negative contrast).

Many of the procedures presently conducted in the field of nuclear medicine involve radiopharmaceuticals which provide diagnostic images of blood flow (perfusion) in the major organs and in tumors. The regional uptake of these radiopharmaceuticals within the

organ of interest is proportional to flow; high flow regions will display the highest concentration of radiopharmaceutical, while regions of little or no flow have relatively low concentrations. Diagnostic images showing these regional differences are useful in identifying areas of poor perfusion, but do not provide metabolic information of the state of the tissue within the region of apparently low perfusion.

It is well known that tumors often have regions within their mass which are hypoxic. These result when the rapid growth of the tumor is not matched by the extension of tumor vasculature. A radiopharmaceutical which localizes preferentially within regions of hypoxia could be used to provide images which are useful in the diagnosis and management of therapy of tumors, as suggested by Champman, "Measurement of Tumor Hypoxia by Invasive and Non-Invasive Procedures--A Review of Recent Clinical Studies", Radiother. Oncol. (1991), 20(S1), 13-19. Additionally, a compound which localizes within the hypoxic region of tumors, but is labeled with a radionuclide with suitable alpha- or beta-emissions could be used for the internal radiotherapy of tumors. In the brain or heart, hypoxia typically follows ischemic episodes produced by, for example, arterial occlusions or by a combination of increased demand and insufficient flow.

However, many radionuclides are less than ideal for routine clinical use. For example, the positron-emitting isotopes (such as $^{18}$F) are cyclotron-produced and short-lived, thus requiring that isotope production, radiochemical synthesis, and diagnostic imaging be performed at a single site or region. The costs of procedures based on positron-emitting isotopes are very high, and there are very few of these centers worldwide. While $^{123}$I-radiopharmaceuticals may be used with widely-available gamma camera imaging systems, $^{123}$I has a 13 hour half-life (which restricts the distribution of radiopharmaceuticals based on this isotope) and is expensive to produce. Nitroimidazoles labeled with $^{3}$H are not suitable for in vivo clinical imaging and can be used for basic research studies only.

A number of factors must be considered for optimal radioimaging in humans. To maximize the efficiency of detection, a radionuclide that emits gamma energy in the 100 to 200 keV range is preferred. To minimize the absorbed radiation dose to the patient, the physical half-life of the radionuclide should be as short as the imaging procedure will allow. To allow for examinations to be performed on any day and at any time of the day, it is advantageous to have a source of the radionuclide always available at the clinical site.

A variety of radionuclides are known to be useful for radioimaging, including Ga-67, Tc-99m, In-111, I-123, and I-131. The preferred radioisotope for medical imaging is Tc-99m. Its 140 keV gamma-photon is ideal for use with widely-available gamma cameras. It has a short (6 hour) half life, which is desirable when considering patient dosimetry. Tc-99m is readily available at relatively low cost through commercially-produced $^{99}$Mo/Tc-99m generator systems. As a result, over 80% of all radionuclide imaging studies conducted worldwide utilize Tc-99m. See generally Reedijk J. "Medicinal Applications of heavy-metal compounds" Curr. Opin. Chem. Biol. (1999) 3(2): 236-240; and Hom, R. K., Katzenellenbogen, J. A. "Technetium-99m-labeled receptor-specific small-molecule radiopharmaceuticals: recent developments and encouraging results" Nuc. Med. and Biol. (1997) 24: 485-498. These advantages, coupled with the fact that Single Photon Emission Computed Tomography cameras are optimized for the 140 keV energy of Tc-99m, clearly demonstrate the superiority of Tc-99m-labeled imaging agents.

Recently, a new Tc(I) labeling system has been developed. Aberto, R., Schibli, R., Egli, A., Schubiger, A. P., Abram, U., Kaden, T. A. "A Novel Organometallic Aqua Complex of Technetium for the Labeling of Biomolecules: Synthesis of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ from $[^{99m}TcO_4]^-$ in Aqueous Solution and Its Reaction with a Bifunctional Ligand" J. Am. Chem. Soc. (1998), 120: 7987-7988; and Alberto, R., Schibli, R., Daniela, A., Schubiger, A. P., Abram, U., Abram, S., Kaden, T. A. "Application of technetium and rhenium carbonyl chemistry to nuclear medicine -- Preparation of $[Net_4]_2[TcCl_3(CO)_3]$ from $[NBu_4][TcO_4]$ and structure of $[NEt_4][Tc_2(u-Cl)_3(CO)_6]$; structures of the model complexes $[NEt_4][Re_2(u-OEt)_2(u-OAc)(CO)_6]$ and $[ReBr(\{-CH_2S(CH_2)_2Cl\}_2(CO)_3]$" Transition Met. Chem. (1997), 22: 597-601. This system takes advantage of the organometallic Tc(I) carbonyl chemistry. Importantly, the chemistry of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ has been elucidated and simplified to the point where the methods are routine and offer a practical alternative to the currently employed Tc(V) chemistry. In contrast to the highly reactive Tc(V)-oxo cores, where the chemistry is sometimes unpredictable and includes labeling cleanup steps, the Tc(I) method offers an attractive labeling alternative. However, unlike the Tc(V)-oxo core, the Tc(I)(CO)$_3^+$ core limits the number of possible coordination geometries available for Tc due to the presence of the three carbonyl groups. The facial arrangement of carbonyl ligands around the metal center also impose steric constraints on the binding possibilities of the remaining three sites.

Moreover, the $[^{99m}Tc(OH_2)_3(CO)_3]^+$ complex can be readily prepared in saline under

1 atm of carbon monoxide (CO). This water and air stable Tc(I) complex is a practical precursor to highly inert Tc(I) complexes, due in part to the $d^6$ electron configuration of the metal center. As already pointed out, the preparation of the organometallic tris(aquo) ion is simple and straightforward, allowing for convenient manipulation and product formation.

5      Substitution of the labile $H_2O$ ligands has been shown to leave the $Tc(CO)_3^+$ core intact. This stable core has the additional advantage of being smaller and less polar than the routinely employed Tc(V)-oxo systems. This characteristic could be advantageous in biologically relevant systems where the addition of the metal center effects the size, shape, and potentially the bioactivity of the compounds.

10      Although various chelators are currently employed in the binding of tectnetium, all of these tracers suffer from one or more disadvantages which render them less than ideal: HYNIC requires coligands; MAG3 may be only used with the Tc(V)-oxo species; EDTA/DTPA is used primarily with Tc(V)-oxo and its ability to retain label is poor. Hence, additional Technetium-99m chelators are needed. Novel radiolabeled chelators that

15      display rapid, efficient labeling and demonstrate superior labeling retention for both Tc(V)-oxo and Tc(I)-tricarbonyl cores without the use of coligands are attractive candidates for clinical evaluation as potential chelators for biologically relevant molecules.

In addtion to radioimaging, discussed above, another widely employed technique for visualizing specific biological processes is fluorescence microscopy. Fluorescence

20      microscopy is a powerful tool for looking at the distribution of fluorescent probes in vitro and for studying the dynamics of protein motion. The high spatial resolution of this method allows for accurate localization of the probe within a cell (while, as explained above, radioimaging allows for the study of molecular processes in vivo by detecting the distribution of a molecule tagged with a radionuclide). Functional radioimaging studies can

25      be carried out in humans or in animals non-invasively, which in the latter case, are now often performed on commercially available scanners that are designed specifically for producing high resolution images (mm scale) of animals.

There have been an increasing number of reports where compounds used to target radionuclides to specific receptors for in vivo imaging studies, have also been labeled with

30      fluorescent probes so that the localization of the bioconjugate can be determined at the cellular level, which is beyond the resolution of radioimaging techniques like positron emission tomography (PET) and single photon emission computed tomography (SPECT).

Gallazzi, F.; Wang, Y.; Jia, F.; Shenoy, N.; Landon, L.A.; Hannink, M.; Lever, S.Z.; Lewis, M.R. "Synthesis of Radiometal-Labeled and Fluorescent Cell-Permeating Peptide-PNA Conjugates for Targeting the *bcl-2* Proto-oncogene" Bioconjugate Chem. (2003), 14(6): 1083-1095; and (b) Bullok, K.E.; Dyszlewski, M.; Prior, J.L.; Pica, C.M.; Sharma, V.;

5          Piwnica-Worms, D. "Characterization of Novel Histidine-Tagged Tat-Peptide Complexes Dual-Labeled with $^{99m}$Tc-Tricarbonyl and Fluorescein for Scintigraphy and Fluorescence Microscopy" Bioconjugate Chem. (2002), 12: 1226. Unfortunately, the structures of common fluorescent probes and radionuclide prosthetic groups are significantly different which introduces a potential source of error when comparing data from in vitro and in vivo

10        experiments. The optimal system would be one in which the fluorescent and radioactive prosthetic groups are iso-structural.

Technetium-99m is the most widely used radionuclide in diagnostic medicine owing to its ideal nuclear properties, low cost and widespread availability. There are a significant number of different Tc complexes that are used clinically including agents that are designed

15        to image bone metastases and myocardial function. Re(I) complexes on the other hand, have been used to prepare luminescent probes. These complexes are particularly useful for studying biological processes in vitro because of their long-lifetime, polarized emission and large Stoke's shift which overcomes issues of self-quenching. Based on the fact that the coordination chemistry of the two congeners is very similar, it should be possible to design

20        a ligand that forms a fluorescent Re complex and a stable $^{99m}$Tc complex. Such a system, which should also possess the ability to be linked to a targeting agent, would allow images obtained on a fluorescent microscope to be directly correlated with in vivo imaging studies.

### *Summary of the Invention*

25        Generally, this invention relates to complexes of technetium (Tc) and rhenium (Re) with various heteroaromatic ligands, *e.g.*, quinolinyl and isoquinolinyl ligands, and their use in fluoresence and radioimaging for a variety of clinical diagnostic applications , as well as radiopharmaceuticals for therapeutic applications. Another aspect of the invention relates to quniolinyl and isoquinolinyl ligands that form a portion of the aforementioned

30        complexes. Methods for the preparation of the technetium and rhenium complexes are also described. Another aspect of the invention relates to quinolinyl and isoquinolinyl ligands

based on derivatized lysine, alanine and bis-amino acids for conjugation to small peptides by solid phase synthetic methods. Additionally, the invention relates to methods for imaging regions of a mammal using the complexes of the invention.

5                                         *Brief Description of Drawings*

**Figure 1** depicts certain novel bifunctional chelates (**L1-L5**).

**Figure 2** depicts the integration of the bifunctional chelate into the peptide sequence.

**Figure 3** depicts the structure of [Re(CO)$_3$(**L1a**-gly)].

10          **Figure 4** depicts previously reported single amino acid chelate (SAAC) **1** and the synthesis of compound **4**.

**Figure 5** depicts (A) an absorbance spectra for compound **4a** (40 μM solution in 5% chloroform 95% ethylene glycol); (B) an emission spectra for compound **4a** in 100% ethylene glycol (under nitrogen and air) or chloroform (under nitrogen or air); and (C) the

15          steady state anisotropy of **4a** as a function of excitation wavelength; excitation was collected in 2 nm increments with an emission wavelength of 590 nm and an integration time of 3 sec, and bandpasses of 5 nm.

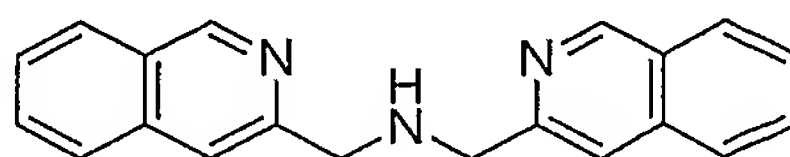*Detailed Description of the Invention*

20     *1. Overview*

We have previously described a class of technetium and rhenium chelating agents based on the derivatization of di(pyridinemethyl)amine (DMPA), a compound that has demonstrated affinity for binding technetium. *See* United States Patent Application Publication 2003/0235843 A1. Specifically therein we described the synthesis,

25     radiolabeling, rhenium modeling, and testing of novel radioactive dimethylpyridine derivatives as bifunctional chelators which demonstrate a high binding affinity for Tc-99m, and have been derivatized to become biochemical probes for the assessment of a variety of biological processes, ranging from infection to cancer diagnosis. In one aspect of that invention, we developed Tc(I) binding ligand **1** (Figure 4). Ligand **1**, which was refered to

as a bifunctional chelate, can form an inert complex with a $\{M(CO)_3\}^{+1}$ core (wherein M is, for example, Re or $^{99m}Tc$).

Herein we describe the preparation of bifunctional chelates whose $\{M(CO)_3\}^{+1}$ complexes are fluorescent. In a preferred embodiement the bifunctional chelate is a
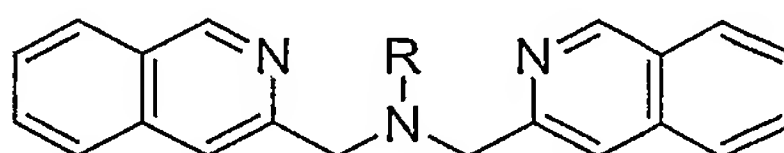
5   $M(CO)_3$-bis(isoquinolinyl) complex.

One aspect of the present invention involves the use of di(isoquinolinemethyl)amine as a tridentate ligand for radionuclides. The ligand demonstrates remarkable ability to rapidly bind both Tc(V)-oxo and Tc(I)-tricarbonyl cores. Notably, the neutral ligand utilizes all three nitrogens as donors to chelate the metal center.
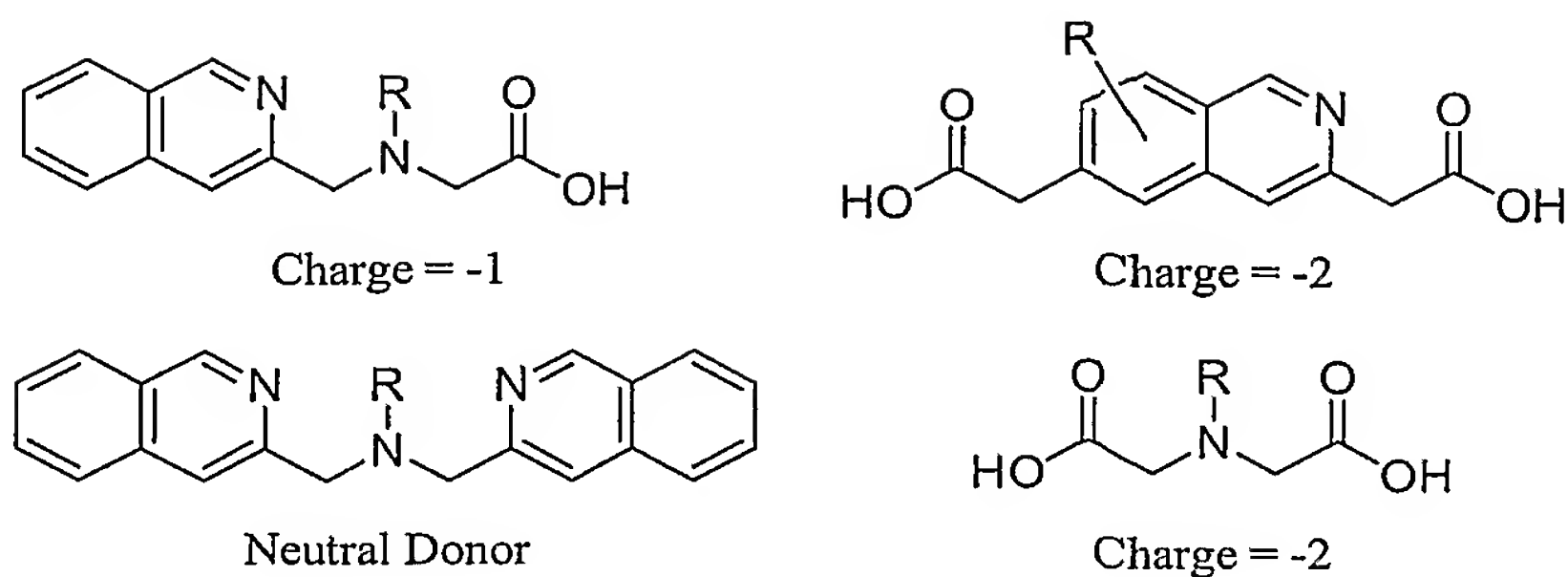
10



Moreover, a biologically relevant molecule, e.g., a peptide or DAT ligand, can be covalently linked to the central nitrogen of the ligand without interfering with the ligand's ability to chelate the radionuclide. The following drawing depicts this embodiment,

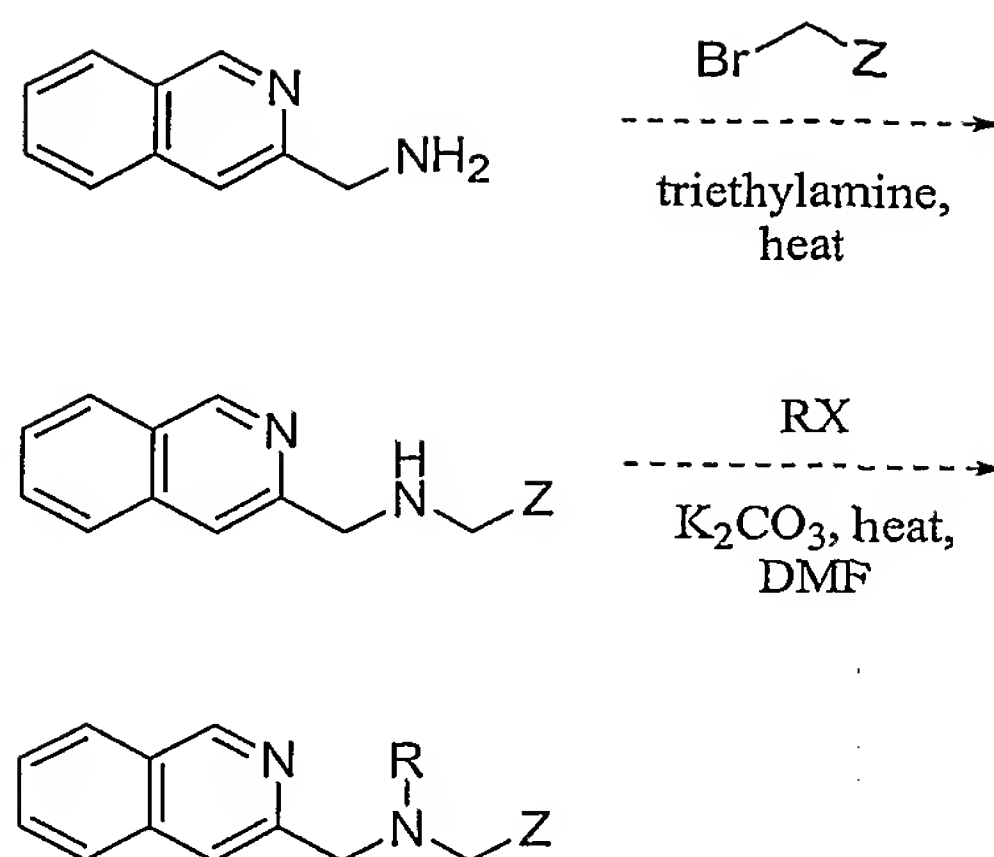15   wherein R represents a biologically relevant molecule.



Chelators based on these ligands serve as neutral, i.e., uncharged, tridentate (N-N-N)

20   donors for both the Tc(V)-oxo and Tc(I)-tricarbonyl cores. However, ligands can be prepared that are cationic or anionic, e.g., depending on the charge of the group attached to the central nitrogen in the structures above. Additionally, the various classes of ligands shown below may be used with the Tc(I)-tricarbonyl core. Some examples are presented below.

25

Charge = -1

Charge = -2

Neutral Donor

Charge = -2

Another aspect of the present invention relates to development of novel Tc-99m labeled analogs, and evaluation of their potential as myocardial blood flow imaging agents

5   (e.g., investigation as potential heart imaging agents in rats). The rationale behind these studies is that the chelate is small, lipophilic, and potentially cationic at physiological pH, all of which are characteristics of effective blood flow agents.

A major concern when designing a chelated-Tc-99m labeled pharmaceutical is that the inclusion of the Tc-ligand in the carrier molecule should not drastically alter the

10   biological behavior of the carrier. *See* Hom, R. K., Katzenellenbogen, J. A. "Technetium-99m-labeled receptor-specific small-molecule radiopharmaceuticals: recent developments and encouraging results" Nuc. Med. and Biol. (1997) 24: 485-498. In these labeling approaches, the chelated radionuclide is bound to the bio-molecule via a pendant chain distant to the receptor-binding site. Advantages of this design include the ability to change

15   the length and location of the pendant chain, as well as the ability to vary chelating moieties. By adopting these ideas one may quickly synthesize a series of versatile chelators that could be functionalized with various biological molecules. Scheme 1 depicts the synthesis of various derivatives.
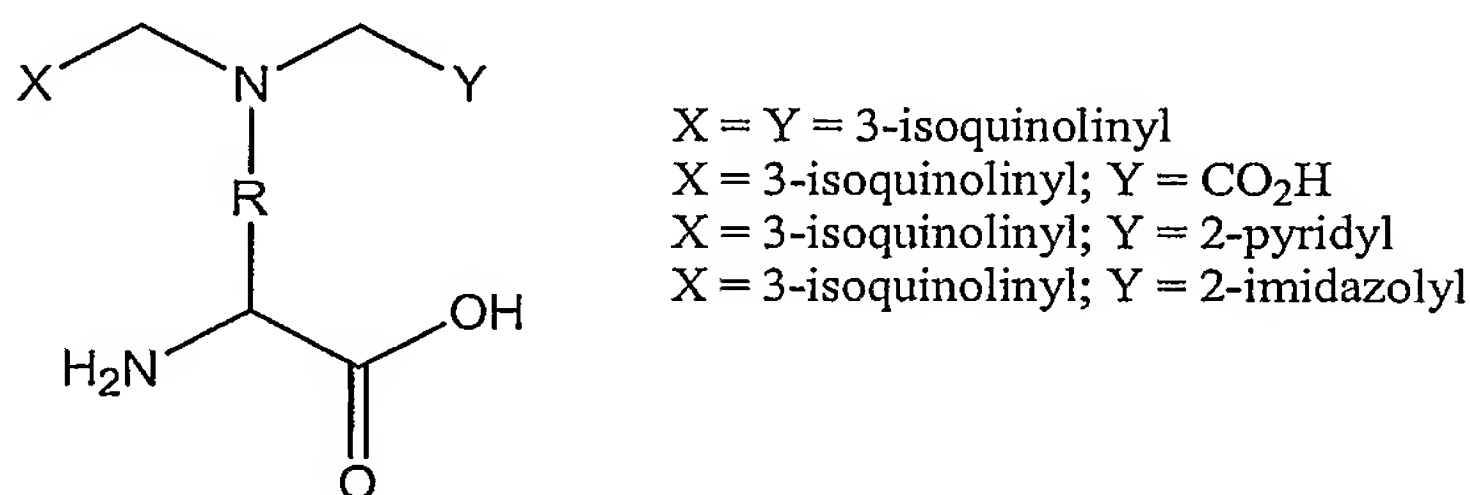
**Scheme 1.** Synthesis of various derivatives (Z is heterocycle or ester)

This work lead to the design of bifunctional chelators constructed from amino acids,
5   so as to provide a donor set for effective coordination of Tc(I) and a linker group for
attachment to peptide units. The significance of this ligand design is that the bifunctional
chelators may be developed as reagents for direct incorporation into conventional solid
phase peptide syntheses (SPPS), thus exploiting the considerable advantages in purity, cost,
scale and design afforded by SPPS.

10   In a preliminary study, the related alanine derivative (bis-2-pyridylmethylamino-
ethylcarboxylic acid, **L3a**) was prepared by the methods described below. The Tc(I)
complex of **L3a** [Tc(CO)₃(**L3a**)] was prepared in nearly quantitative yield, as well as an
unusual material exhibiting the rhenium(IV)-trichloride core [ReCl₃(**L3a**-ethylester)]. The
facile preparations of these model compounds suggested that a family of bifunctional
15   chelators, derived from simple amino acids or bis-amino acids could be developed, which
through suitable manipulation of the ligand donor groups can provide neutral, cationic or
anionic Tc(I) complexes.

One goal of the present invention is to develop a family of bifunctional chelators
based on quinoline or isoquinoline and/or carboxylate derivatized amino acids or bis-amino
20   acids for conjugation to small peptides by solid phase synthetic methods. To achieve this,
lysine, alanine, aminoalanine and a series of bis amino acids are modified to incorporate a
tridentate chelation terminus, as well as a terminus for conjugation to small peptides
exploiting solid phase synthesis. The optimal design of the tether is also investigated. In

certain embodiments, the present invention relates to amino acids, e.g., alpha-amino acids, bearing covalently linked bifunctional chelators for radionuclides, e.g., technetium. For example, the present invention relates to compounds represented by scheme 2, wherein R represents a covalent tether, e.g., a butylene linker as in Lys, between the alpha carbon of

5    the alpha-amino acid and a bifunctional chelator of the instant invention for a radionuclide. Amino acids, such as those in scheme 2, bearing a bifunctional chelator for a radionuclide may be used in place of natural amino acids in any of the methods of oligopeptide, polypeptide or protein synthesis, including the methods of automated protein synthesis.
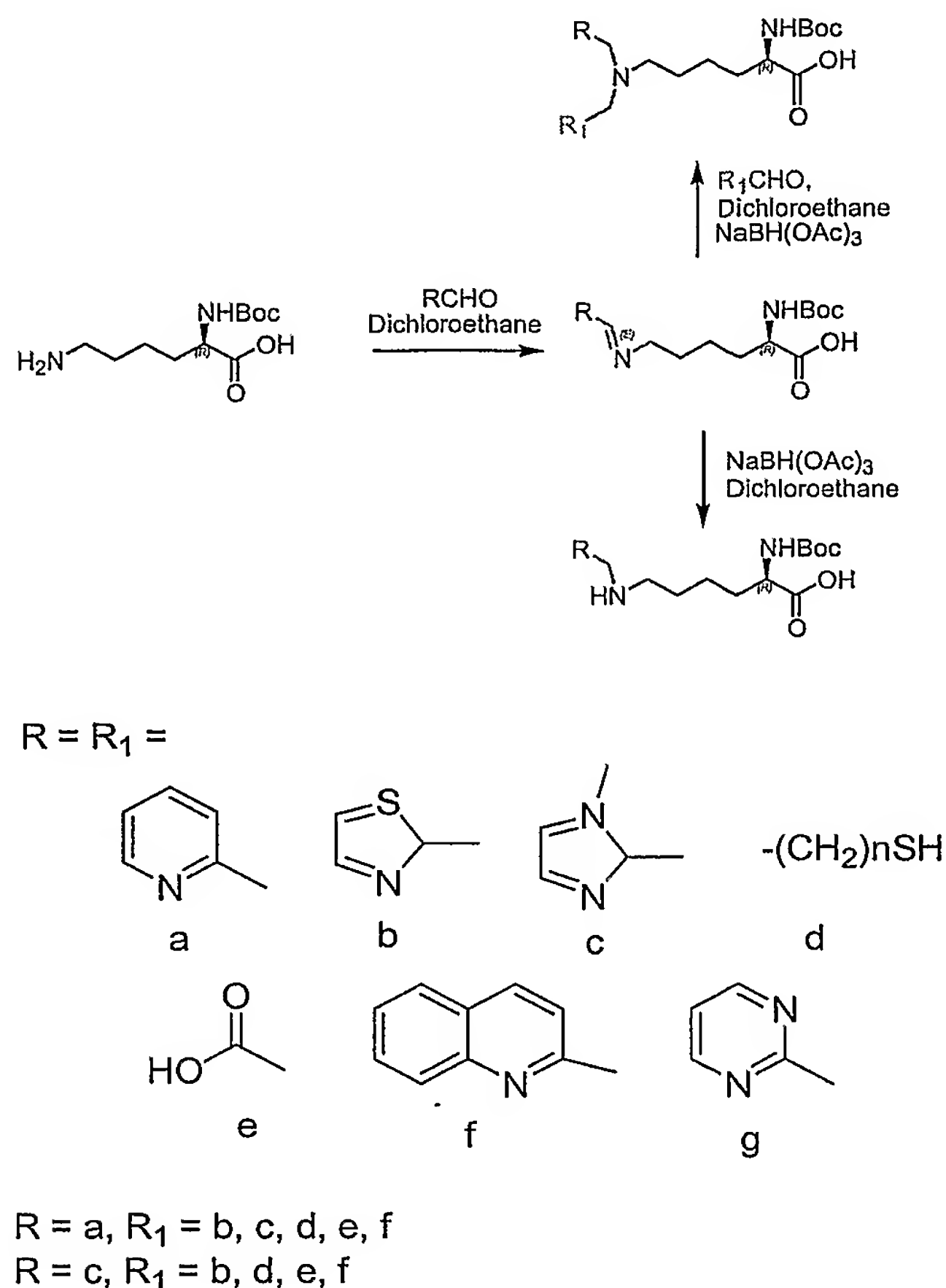
X = Y = 3-isoquinolinyl
X = 3-isoquinolinyl; Y = CO$_2$H
X = 3-isoquinolinyl; Y = 2-pyridyl
X = 3-isoquinolinyl; Y = 2-imidazolyl

10                        **Scheme 2.** Amino acids bearing a bifunctional chelator

*2. Design and Synthesis of Bifunctional Chelates*

The "organometallic approach" for functionalization and radiolabeling of target specific biomolecules, pioneered by Jaouen, has received considerable attention in recent years. Salmain, M.; Gunn, M.; Gorfe, A.; Top, S.; Jaouen, G. *Bioconjugate Chem.* **1993**, *4,*

15   425. In particular, Tc(I)- and Re(I)- tricarbonyl complexes are ideal candidates for the labeling of receptor avid biomolecules in terms of reduced size and kinetic inertness of their complexes. The $\{M(CO)_3\}^{+1}$ core exhibits particular affinity for nitrogen and oxygen donor ligands and forms robust complexes with such tridentate N,O donor ligands of the general type $[M(CO)_3(N_xO_{3-x})]$, where $N_xO_{3-x}$ is the tridentate chelator. This observation provides

20   the conceptual starting point for the design of our bifunctional chelates for peptide labeling.

As illustrated in Figure 1, certain novel bifunctional chelates (**L1-L5**) can be derived from lysine, alanine, aminoalanine or bis-amino acids. Since both the identity of the donor groups and the amino acid backbone can be readily modified, the chelator and the linker termini may be optimized for $^{99m}$Tc coordination and peptide conjugation, respectively.

25   Furthermore, by modifying the identities of the chelating donor groups, neutral, anionic and cationic complexes of general types [M(CO)$_3$(**L1a**)], [M(CO)$_3$(**L1b**)]$^-$, [M(CO)$_3$(**L1c**)]$^+$ and

[M(CO)$_3$(**L1d**)]$^+$ may be prepared for different applications. *See* Figure 1. Respresentative ligand syntheses are detailed below for Scheme 3, illustrating the direct and facile methodology.



R = R$_1$ =

a     b     c     -(CH$_2$)nSH
                        d

                 e     f     g

R = a, R$_1$ = b, c, d, e, f
R = c, R$_1$ = b, d, e, f

**Scheme 3.** A general synthetic scheme for the preparation of mono-, di- and mixed alkylated SAACs, L$_x$. The method may be adapted to any amino acid or amino acid analogue.

Conventional solid phase synthesis can be exploited to prepare the peptide conjugate. Bodansky, M., *Principles of Peptide Synthesis,* Springer-Verlag: Berlin, **1984**; and Bodansky, M.; Bodansky, A., *The Practice of Peptide Synthesis,* Springer-Verlag: Berlin, **1984**. The peptide chain can be constructed using FMOC protocols and capped with a BOC protecting group (Figure 2). The bifunctional chelator (BFC) may now be introduced to provide a pendant peptide-BFC design. Alternatively, the bis-amino acid based BFCs may be incorporated into the peptide sequence to provide a variant of the integrated design concept.

*3. Synthesis of Rhenium Analogs for Structural Characterization*

Many of the properties of the Group VII metals technetium and rhenium are similar. It is anticipated that the metals will demonstrate similar reaction chemistry, which is often the case for the thiol, nitrogen, phosphine and oxo-chemistry of these two metals. Likewise,

5      perrhenate and pertechnetate have very similar reaction behaviors. Rose, D. J., Maresca, K. P., Nicholson, T., Davison, A., Jones, A. G., Babich, J., Fischman, A., Graham, W., DeBord, J. R. D., Zubieta, J. "Synthesis and Characterization of Organohydrazine Complexes of Technetium, Rhenium, and Molybdenum with the {M($\eta$1-HxNNR)($\eta$2-HyNNR)} Core and Their Relationship to Radiolabeled Organohydrazine-Derivatized

10     Chemotactic Peptides with Diagnostic Applications" *Inorg. Chem.* **1998**, *37*, 2701-2716. The similar reductions of the M(VII) oxo species by $SnCl_2$ allowed for easy substitution of the nonradioactive rhenium as a model for the medicinally useful technetium-99m, which routinely uses tin-reduced $^{99m}Tc$. Synthesizing the rhenium-dipyridinemethylamine complexes provided a facile route to structurally characterize the products. The periodic

15     relationship between Tc and Re indicates that Tc-99m radiopharmaceuticals can be designed by modeling analogous rhenium complexes. Nicholson, T., Cook, J., Davison, A., Rose, D. J., Maresca K. P., Zubieta, J. A., Jones, A. G. "The synthesis and characterization of $[MCl_3(N=NC_5H_4NH)(HN=NC_5H_4N)]$ from $[MO_4]^-$ (where M = Re, Tc) organodiazenido, organodiazene-chelate complexes" *Inorg. Chim. Acta* **1996**, *252*, 421-426. The

20     coordination chemistry with ${Re(CO)_3(H_2O)_3}^+$ has produced a number of derivatives including the model compound $[Re(CO)_3(\mathbf{L1a}\text{-gly})]$, shown in Figure 3.

The synthesis of the rhenium analogs followed the established chemistry of the $N_2S_2$ system in forming stable, neutral, rhenium-oxo complexes. Davison A, Jones AG, Orvig C, et al. "A new class of oxotechnetium ($5^+$) chelate complexes containing a $TcON_2S_2$ core"

25     *Inorg. Chem.* **1981**, *20*, 1629-1631; Kung HF, Guo Y-Z, Mach RH, et al. "New Tc-99 complexes based on $N_2S_2$ ligands" *J. Nucl. Med.* **1986**, *27*, 1051 (abstr.); Kung HF, Molnar M, Billings J, et al: "Synthesis and biodistribution of neutral lipid-soluble Tc-99m complexes that cross the blood-brain barrier" *J. Nucl. Med.* **1984**, *25*, 326-332; and Kung HF, Yu CC, Billings J, et al: "Synthesis of new bis(aminoethanethiol) (BAT) derivatives:

30     Possible ligands for $^{99m}Tc$ brain imaging agents" *J. Med. Chem.* **1985**, *28*, 1280-1284. Our $N_3$ system, with three nitrogen donors forms a predictablable metal-complex with an overall net charge of zero. The synthesis of the Re(III) complexes was accomplished by reacting

[TBA][ReOBr₄(OPPh₃)] with the appropriate ligand in the ratio of 1:1.2 in 10 mL of methanol and three equivalents of NEt₃ as base. The reaction was allowed to reflux for roughly ½ hour. After cooling, the reaction products were be purified using a small column using the method established by Spies and co-workers. Spies, H., Fietz, T., Glaser, M.,

5    Pietzsch, H.-J., Johannsen, B. In "Technetium and Rhenium in Chemistry and Nuclear Medicine 3", Nicollini, M., Bandoli, G., Mazzi, U., eds., Padova, Italy, **1995**, 4, 243. Alternatively, the rhenium (V) starting material [ReOCl₃(PPh₃)₂] may be employed as the potential rhenium starting material. This versatile material has proven successful in the past for dealing with nitrogen and sulfur donor atoms. Maresca, K. P., Femia, F. J., Bonavia, G.

10   H., Babich, J. W., Zubieta, J. "Cationic comples of the '3+1' oxorhenium-thiolate complexes" *Inorganic Chemistry Acta* **2000**, *297*, 98-105; and Maresca, K. P., Rose, D. J., Zubieta, J. "Synthesis and charaterization of a binuclear rhenium nitropyrazole" *Inorganica Chimica Acta* **1997**, *260*, 83-88. The synthesized rhenium-DPMA complexes have been run through a HPLC column for separation and purification purposes following the

15   procedures described for the technetium complexes. The complexes were then analyzed by elemental analysis, infrared spectroscopy, mass spectroscopy, and NMR spectroscopy.

The Re(I)(CO)₃⁺ system displays similar reaction chemistry to that of the Tc-99m tricarbonyl core. The use of [NEt₄]₂[ReBr₃(CO)₃], as the starting material leads to easy formation of the *fac*-Re(CO)₃(L)₃ core. The [NEt₄]₂[ReBr₃(CO)₃] is readily derived from

20   the [ReBr(CO)₅]. The synthesis of the Re(I) complexes has been accomplished by reacting [NEt₄]₂[ReBr₃(CO)₃] with the appropriate DPMA ligand in the ratio of 1: 1.2 in 10 mL of H₂O and three equivalents of NEt₃ as base. The reaction was allowed to heat at 80 °C for 4 hours. After cooling, the reaction products were purified using a small column using the method established by Alberto and coworkers. Spies, H., Fietz, T., Glaser, M., Pietzsch,

25   H.-J., Johannsen, B. In "Technetium and Rhenium in Chemistry and Nuclear Medicine 3", Nicollini, M., Bandoli, G., Mazzi, U., eds., Padova, Italy, **1995**, 4, 243. This versatile material has proven successful in the past for dealing with nitrogen and oxygen donor atoms. The synthesized rhenium-DPMA complexes were then run through a HPLC column for separation and purification purposes, following the procedures previously described for

30   the technetium complexes. Next, the complexes were analyzed by: elemental analysis, infrared spectroscopy, mass spectroscopy, and NMR spectroscopy.

*4. Preparation and Charaterization of Fluorescent SAAC of Tc and Re*

Recently we reported the synthesis of a Tc(I) binding ligand which is a single amino acid chelate (SAAC) (**1**, Figure 4). As shown herein, SAACs can form inert complex with the $\{M(CO)_3\}^{+1}$ core (M = Re, $^{99m}$Tc) and can be incorporated into peptides as if it were a natural amino acid. To prepare a SAAC type ligand whose Re complex is fluorescent, while retaining the ability to bind $^{99m}$Tc, N-α-Fmoc-L-lysine was reacted with isoquinoline aldehyde in the presence of Na(OAc)$_3$BH to give the bifunctional ligand **3** (Figure 4). The desired product, which can be produced in multi-gram quantities, was isolated in excellent yield following column chromatography. The Re complex **4a** was synthesized by reacting **3** with [NEt$_4$]$_2$[Re(CO)$_3$Br$_3$]. The complex was isolated as the TEA salt by column chromatography.

Figure 5a shows the absorbance spectrum of compound **4a** from 250 to 700 nm in 5% chloroform 95% ethylene glycol. The rhenium compound has appreciable absorbance in the UV and blue regions of the UV-visible spectrum with a peak absorbance at 301 nm. The extinction coefficients for compound **4a** were 13,200 M$^{-1}$ cm$^{-1}$ at 301 nm and 2250 M$^{-1}$ cm$^{-1}$ at 366 nm. Emission was monitored from 400 to 700 nm with excitation wavelength of 366 nm in 1 nm increments with an integration time of 0.25 sec and bandwidths of 5 nm. Spectra were acquired in ethylene glycol and chloroform solutions both in the presence of air and under nitrogen (Figure 5b). The rhenium probe has two distinct transitions giving rise to peak fluorescence intensities at 425 and 580 nm.

To assess whether the rhenium probe may be suitable for polarization based fluorescence assays, the steady state fluorescence anisotropy was measured as a function of excitation wavelength (Figure 5c). A solution of compound **4a** was prepared in 100% ethylene glycol and cooled to − 20 °C to slow the rotational movement of the fluorescent molecule. Under these conditions the fluorescence anisotropy increases to a limiting anisotropy of 0.35 at a wavelength of 424 nm.

Compound **4a** has a number of attractive properties as a ligand for luminescence studies. The probe absorbs in the ultraviolet region spectrum, and has an emission maximum at approximately 585 nm which avoids complications that arise in physiological studies due to cell auto-fluorescence. In similar respects, the probe has an extremely long lifetime ranging between 1 to 16 μs. This is also beneficial for physiological studies because

cell auto-fluorescence occurs on the nanosecond time scale, and can therefore be eliminated using time-gating techniques so long as the probe under investigation has a sufficiently long lifetime. An additional advantage to compound **4a** is that it exhibits fluorescence anisotropy making it useful for small molecule/cell receptor or protein/protein binding studies in which
5   the rotational mobility of the probe can be monitored as a function of binding. The one drawback to compound 4a is that it has a low quantum yield, which ranges from 0.0027 in chloroform in the presence of air to 0.0145 in ethylene glycol under nitrogen.

To determine if the SAACQ ligand forms a stable $^{99m}$Tc complex, compound **3** was added to $[Tc(CO)_3(OH_2)_3]^+$, which was prepared by adding $TcO_4^-$ to a commercially
10  available carbonyl labeling kit. The desired product **4b** was isolated in high yield even when very small amounts of the ligand were used. The stability of the complex to transchelation was investigated by incubating two separate samples of **4b** with 1000 fold excess of cysteine and histidine in PBS buffer heated to 37 °C. After 24 hours there was almost no sign of degradation which clearly indicates that compound **4b** is suitably robust for use in
15  vivo.

As explained previously, because the SAACQ ligand and the SAACQ-Re complex are amino acid analogues they can be readily incorporated into a peptide at any position using a conventional peptide synthesizer. To demonstrate this feature the SAACQ and SAACQ-Re complexes were incorporated within fMLF; a targeting sequence which has
20  been used to guide radionuclides to the formyl peptide receptor as a means of imaging sites of infection and inflammation. The peptides fMLF(SAACQ)Gly (**5**) and fMLF[(SAACQ-Re(CO)_3)^+]G (**6**) were prepared following standard FMOC synthetic methods using a glycine loaded SASRIN resin and HBTU-HOBt as the coupling agent. The peptides were isolated using a standard cleavage cocktail (94% TFA, 2% EDT, 2% TIS and 2% water) and
25  the products purified by HPLC. The HPLC purification was needed as a result of epimerization of the methionine residue which is known to occur under normal solid-phase synthesis conditions. It was not a consequence of introducing the SAACQ ligand or the SAACQ-Re complex.

The affinity of **5** and **6** for the formyl peptide receptor was determined by flow
30  cytometry using fluorescein labeled fNLFNTK as the reference ligand. Compounds **5** and **6** showed $K_d$ values of $11 \pm 3$ nm and $27 \pm 13$ nm which is comparable to that for the parent targeting agent and for fMLF(SAAC)G and fMLF[(SAAC-Re(CO)_3)^+]G.

*5. Methods of Use*

We have also explored extensively the use as ligands for metal tricarbonyls, *e.g.*, Re and Tc tricarbonyls, of protected and unprotected versions of [ε-{*N,N*-di(pyridyl-2-methyl)}α-(fmoc)lysine] (Fmoc-DpK). The choice of the tridentate DpK for the

5      exploration of a single amino acid chelate was based on the excellent RCP and RCY, and the potential to prepare radiopharmaceutical kits. The pyridine-2-methylamine was easily derivatized into the amino acid. The biodistribution results showed [$^{99m}$Tc(CO)$_3$(DpK)] having rapid blood clearance with %ID/g = 0.6 at 5 minutes to %ID/g = 0.07 by 30 minutes.

This approach enables the creation of libraries containing the {M(CO)$_3$}$^{1+}$ core. We

10     have begun to define the biological fate of the $^{99m}$Tc-bis(heteroaryl) complexes, allowing us to compare a series of future tridentate analogs. The dipyridine labeling proceeded in high yield and was stable to excess histidine and cysteine challenges for more than 18 hours. Biodistribution studies showed major accumulation in kidney and liver only, at early timepoints. Activity decreased in all tissues as a function of time, except in the GI tract,

15     which increased with time. These experiments suggest that dipyridine is a potential enabling technology for the labeling of important biomolecules.

With the approach reported here, it is possible to prepare virtually any small peptide-conjugate with a synthon that can be used as a fluorescent probe and as a tracer for radioimaging studies. The ability to directly correlate *in vitro* and *in vivo* imaging studies

20     goes a long way towards bridging the gap between work in isolated cells and studies carried out in living models. This invention will have a significant impact on biochemical research and on the radiopharmaceutical and pharmaceutical development processes, where, particularly in the latter arena, molecular imaging, both *in vitro* and *in vivo*, is playing an increasingly important role.

25     *6. Definitions*

For convenience, before further description of the present invention, certain terms employed in the specification, examples, and appended claims are collected here.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element"

30     means one element or more than one element.

The terms "comprise" and "comprising" are used in the inclusive, open sense, meaning that additional elements may be included.

The term "including" is used herein to mean "including but not limited to". "Including" and "including but not limited to" are used interchangeably.

5        The terms "lipophilic group" and "lipophilic moiety" as used herein refer to a group, moiety or substituent that has a greater affinity for non-polar or non-aqueous environments versus polar or aqueous environments. For example, Merriam Webster's online dictionary defines "lipophilic" as "having an affinity for lipids (as fats)." Exemplary lipophilic moieties include aliphatic hydrocarbon radicals, *e.g.*, alkyl radicals, aromatic hydrocarbon 10   radicals, and long-chain acyl radicals; all of them have increasing lipophilicity as the number of constituent carbons increases. In general, addition of a lipophilic moiety to a particular compound will increase the compound's affinity for octanol in the standard octanol/water partition-coefficient-determination protocol; this protocol may be used to gauge a compound's relative hydrophobicity (lipophilicity) and hydrophilicity.

15      The terms "Lewis base" and "Lewis basic" are art-recognized and generally refer to a chemical moiety capable of donating a pair of electrons under certain reaction conditions. It may be possible to characterize a Lewis base as donating a single electron in certain complexes, depending on the identity of the Lewis base and the metal ion, but for most purposes, however, a Lewis base is best understood as a two electron donor. Examples of 20   Lewis basic moieties include uncharged compounds such as alcohols, thiols, and amines, and charged moieties such as alkoxides, thiolates, carbanions, and a variety of other organic anions. In certain examples, a Lewis base may consist of a single atom, such as oxide ($O_2^-$). In certain, less common circumstances, a Lewis base or ligand may be positively charged. A Lewis base, when coordinated to a metal ion, is often referred to as a ligand. Further 25   description of ligands relevant to the present invention is presented herein.

The term "ligand" is art-recognized and refers to a species that interacts in some fashion with another species. In one example, a ligand may be a Lewis base that is capable of forming a coordinate bond with a Lewis Acid. In other examples, a ligand is a species, often organic, that forms a coordinate bond with a metal ion. Ligands, when coordinated to 30   a metal ion, may have a variety of binding modes know to those of skill in the art, which include, for example, terminal (i.e., bound to a single metal ion) and bridging (i.e., one atom

of the Lewis base bound to more than one metal ion).

The term "chelating agent" is art-recognized and refers to a molecule, often an organic one, and often a Lewis base, having two or more unshared electron pairs available for donation to a metal ion. The metal ion is usually coordinated by two or more electron pairs to the chelating agent. The terms, "bidentate chelating agent", "tridentate chelating agent", and "tetradentate chelating agent" are art-recognized and refer to chelating agents having, respectively, two, three, and four electron pairs readily available for simultaneous donation to a metal ion coordinated by the chelating agent. Usually, the electron pairs of a chelating agent forms coordinate bonds with a single metal ion; however, in certain examples, a chelating agent may form coordinate bonds with more than one metal ion, with a variety of binding modes being possible.

The term "coordination" is art-recognized and refers to an interaction in which one multi-electron pair donor coordinatively bonds (is "coordinated") to one metal ion.

The term "complex" is art-recognized and refers to a compound formed by the union of one or more electron-rich and electron-poor molecules or atoms capable of independent existence with one or more electronically poor molecules or atoms, each of which is also capable of independent existence.

The term "tether" is art-recognized and refers to, as used herein, a chemical linking moiety between a metal ion center and another chemical moiety.

The term "amino acid" is art-recognized and refers to all compounds, whether natural or synthetic, which include both an amino functionality and an acid functionality, including amino acid analogs and derivatives.

The term "heteroatom" is art-recognized and refers to an atom of any element other than carbon or hydrogen. Illustrative heteroatoms include boron, nitrogen, oxygen, phosphorus, sulfur and selenium.

The term "alkyl" is art-recognized, and includes saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In certain embodiments, a straight chain or branched chain alkyl has about 30 or fewer carbon atoms in its backbone (e.g., $C_1$-$C_{30}$ for straight chain, $C_3$-$C_{30}$ for branched chain), and

alternatively, about 20 or fewer. Likewise, cycloalkyls have from about 3 to about 10 carbon atoms in their ring structure, and alternatively about 5, 6 or 7 carbons in the ring structure.

Unless the number of carbons is otherwise specified, "lower alkyl" refers to an alkyl group, as defined above, but having from one to about ten carbons, alternatively from one to about six carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths.

The term "aralkyl" is art-recognized and refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

The terms "alkenyl" and "alkynyl" are art-recognized and refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The term "aryl" is art-recognized and refers to 5-, 6- and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, naphthalene, anthracene, pyrene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics." The aromatic ring may be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, -$CF_3$, -CN, or the like. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic, e.g., the other cyclic rings may be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls.

The terms ortho, meta and para are art-recognized and refer to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and ortho-dimethylbenzene are synonymous.

The terms "heterocyclyl", "heteroaryl", or "heterocyclic group" are art-recognized and refer to 3- to about 10-membered ring structures, alternatively 3- to about 7-membered rings, whose ring structures include one to four heteroatoms. Heterocycles may also be
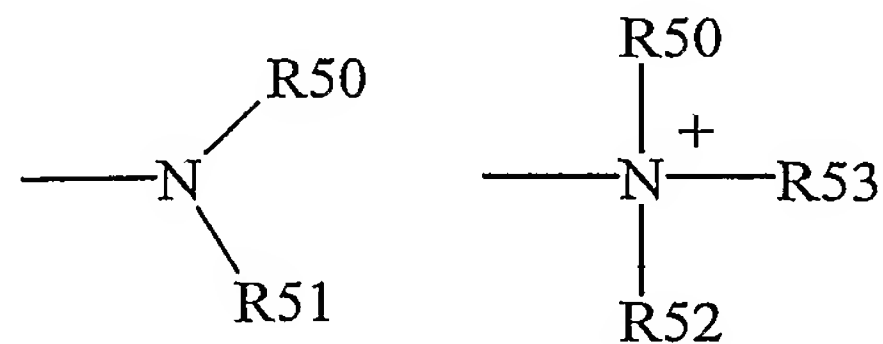
polycycles. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxanthene, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring may be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF$_3$, -CN, or the like.

The terms "polycyclyl" or "polycyclic group" are art-recognized and refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle may be substituted with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF$_3$, -CN, or the like.

The term "carbocycle" is art-recognized and refers to an aromatic or non-aromatic ring in which each atom of the ring is carbon.
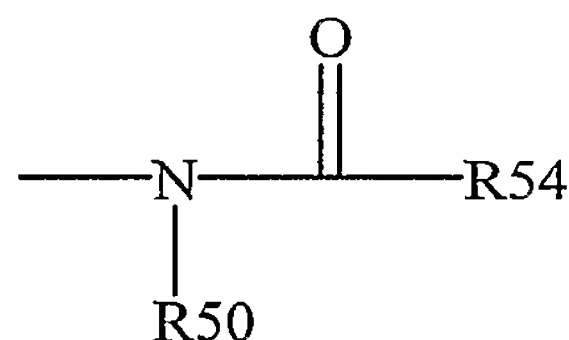
The term "nitro" is art-recognized and refers to -NO$_2$; the term "halogen" is art-recognized and refers to -F, -Cl, -Br or -I; the term "sulfhydryl" is art-recognized and refers to -SH; the term "hydroxyl" means -OH; and the term "sulfonyl" is art-recognized and refers to -SO$_2^-$. "Halide" designates the corresponding anion of the halogens, and "pseudohalide" has the definition set forth on 560 of "Advanced Inorganic Chemistry" by Cotton and Wilkinson.

The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that may be represented by the general formulas:
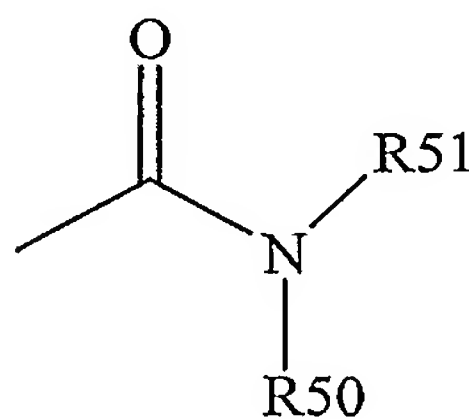
$$\begin{array}{cc} \underset{\underset{R51}{|}}{\overset{\overset{R50}{\diagup}}{-\!\!\!-N}} & \underset{\underset{R52}{|}}{\overset{\overset{R50}{|}}{-\!\!\!-N^+\!\!\!-\!\!\!-R53}} \end{array}$$

5   wherein R50, R51 and R52 each independently represent a hydrogen, an alkyl, an alkenyl, -(CH$_2$)$_m$-R61, or R50 and R51, taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R61 represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocycle or a polycycle; and m is zero or an integer in the range of 1 to 8. In other embodiments, R50 and R51 (and optionally R52) each independently represent a hydrogen, an alkyl, an alkenyl, or -(CH$_2$)$_m$-R61. Thus, the term
10  "alkylamine" includes an amine group, as defined above, having a substituted or unsubstituted alkyl attached thereto, i.e., at least one of R50 and R51 is an alkyl group.

The term "acylamino" is art-recognized and refers to a moiety that may be represented by the general formula:

$$-\!\!\!-\underset{\underset{R50}{|}}{N}\!\!\!-\!\!\!\overset{\overset{\displaystyle O}{\|}}{C}\!\!\!-\!\!\!R54$$

15  wherein R50 is as defined above, and R54 represents a hydrogen, an alkyl, an alkenyl or -(CH$_2$)$_m$-R61, where m and R61 are as defined above.
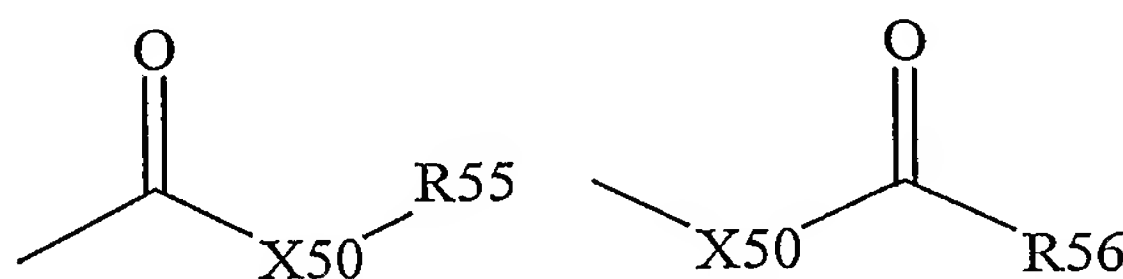
The term "amido" is art recognized as an amino-substituted carbonyl and includes a moiety that may be represented by the general formula:

$$\overset{\overset{\displaystyle O}{\|}}{C}\!\!\!-\!\!\!\underset{\underset{R50}{|}}{N}\!\!\!\overset{R51}{\diagup}$$

20  wherein R50 and R51 are as defined above. Certain embodiments of the amide in the present invention will not include imides which may be unstable.

The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In certain embodiments, the "alkylthio" moiety is represented by one of -S-alkyl, -S-alkenyl, -S-alkynyl, and -S-$(CH_2)_m$-R61, wherein m and R61 are defined above. Representative alkylthio groups include methylthio, ethyl thio, and the like.

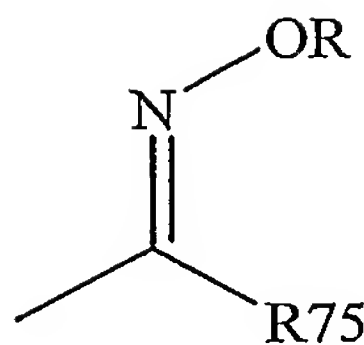The term "carboxyl" is art recognized and includes such moieties as may be represented by the general formulas:

$$\underset{X50}{\overset{O}{\parallel}}\underset{}{C}\!\!-\!\!\overset{R55}{\phantom{O}} \qquad \underset{X50}{\overset{O}{\parallel}}\underset{R56}{C}$$

wherein X50 is a bond or represents an oxygen or a sulfur, and R55 and R56 represents a hydrogen, an alkyl, an alkenyl, -$(CH_2)_m$-R61 or a pharmaceutically acceptable salt, R56 represents a hydrogen, an alkyl, an alkenyl or -$(CH_2)_m$-R61, where m and R61 are defined above. Where X50 is an oxygen and R55 or R56 is not hydrogen, the formula represents an "ester". Where X50 is an oxygen, and R55 is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R55 is a hydrogen, the formula represents a "carboxylic acid". Where X50 is an oxygen, and R56 is hydrogen, the formula represents a "formate". In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a "thiolcarbonyl" group. Where X50 is a sulfur and R55 or R56 is not hydrogen, the formula represents a "thiolester." Where X50 is a sulfur and R55 is hydrogen, the formula represents a "thiolcarboxylic acid." Where X50 is a sulfur and R56 is hydrogen, the formula represents a "thiolformate." On the other hand, where X50 is a bond, and R55 is not hydrogen, the above formula represents a "ketone" group. Where X50 is a bond, and R55 is hydrogen, the above formula represents an "aldehyde" group.

The term "carbamoyl" refers to -O(C=O)NRR', where R and R' are independently H, aliphatic groups, aryl groups or heteroaryl groups.

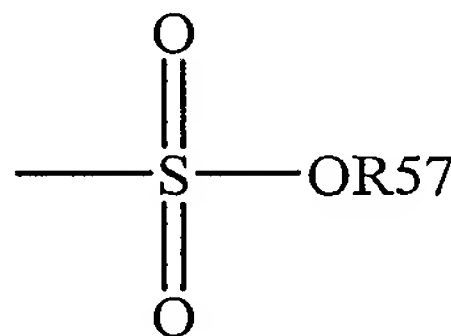The term "oxo" refers to a carbonyl oxygen (=O).

The terms "oxime" and "oxime ether" are art-recognized and refer to moieties that may be represented by the general formula:

$$\underset{\overset{\displaystyle\|}{C}}{\overset{\displaystyle N\diagdown OR}{\phantom{C}}}\diagdown R75$$

wherein R75 is hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, aralkyl, or -(CH$_2$)$_m$-R61. The moiety is an "oxime" when R is H; and it is an "oxime ether" when R is alkyl, cycloalkyl, alkenyl, alkynyl, aryl, aralkyl, or -(CH$_2$)$_m$-R61.

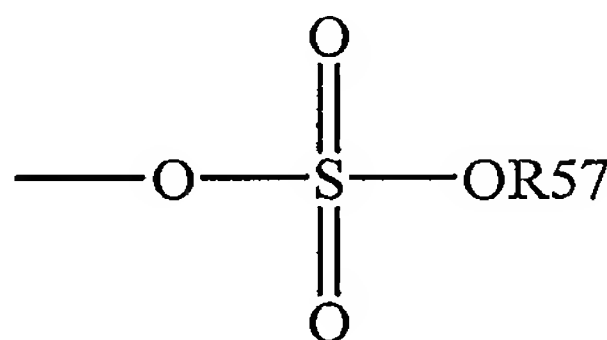5          The terms "alkoxyl" or "alkoxy" are art-recognized and refer to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as may be represented by one of

10      -O-alkyl, -O-alkenyl, -O-alkynyl, -O--(CH$_2$)$_m$-R61, where m and R61 are described above.

The term "sulfonate" is art recognized and refers to a moiety that may be represented by the general formula:

$$\underset{\overset{\displaystyle\|}{O}}{\overset{\displaystyle O}{\overset{\displaystyle\|}{S}}}\!\!-\!\!OR57$$
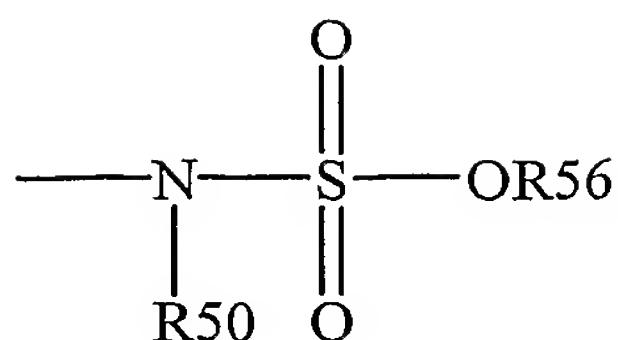
in which R57 is an electron pair, hydrogen, alkyl, cycloalkyl, or aryl.

15          The term "sulfate" is art recognized and includes a moiety that may be represented by the general formula:
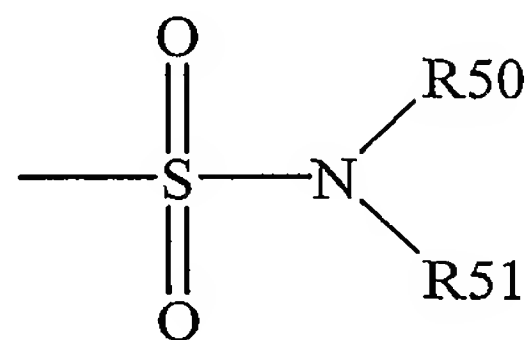
$$-\!\!O\!\!-\!\!\underset{\overset{\displaystyle\|}{O}}{\overset{\displaystyle O}{\overset{\displaystyle\|}{S}}}\!\!-\!\!OR57$$

in which R57 is as defined above.

The term "sulfonamido" is art recognized and includes a moiety that may be

20      represented by the general formula:

- 23 -

$$\overset{\displaystyle O}{\underset{\displaystyle R50 \quad O}{\overset{\displaystyle \|}{-\!\!\!-N\!\!-\!\!\overset{\displaystyle |}{\underset{\displaystyle \|}{S}}\!\!-\!\!OR56}}}$$

in which R50 and R56 are as defined above.

The term "sulfamoyl" is art-recognized and refers to a moiety that may be represented by the general formula:
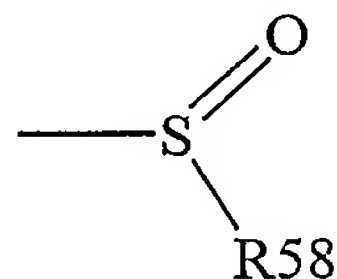
$$-\!\!\!-\overset{\displaystyle O}{\underset{\displaystyle O}{\overset{\displaystyle \|}{\underset{\displaystyle \|}{S}}}}\!\!-\!\!N\overset{\displaystyle R50}{\underset{\displaystyle R51}{}}$$

5

in which R50 and R51 are as defined above.

The term "sulfonyl" is art-recognized and refers to a moiety that may be represented by the general formula:

$$-\!\!\!-\overset{\displaystyle O}{\underset{\displaystyle O}{\overset{\displaystyle \|}{\underset{\displaystyle \|}{S}}}}\!\!-\!\!R58$$
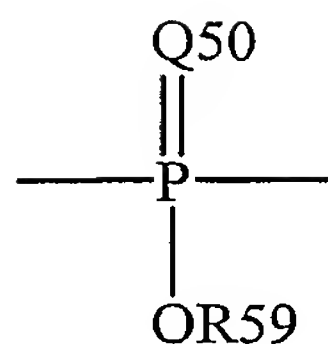
10      in which R58 is one of the following: hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl or heteroaryl.

The term "sulfoxido" is art-recognized and refers to a moiety that may be represented by the general formula:
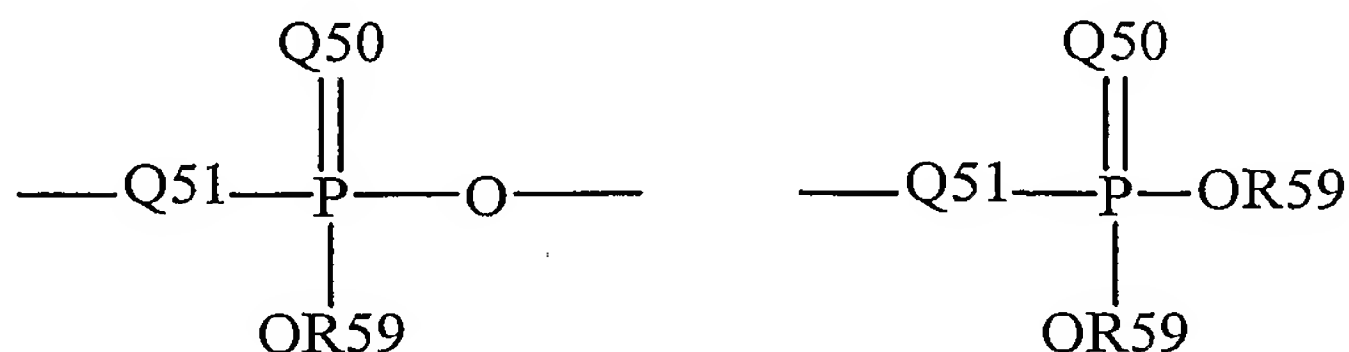
$$-\!\!\!-\overset{\displaystyle O}{\underset{\displaystyle R58}{S}}$$

15     in which R58 is defined above.

The term "phosphoryl" is art-recognized and may in general be represented by the formula:

$$
\begin{array}{c}
Q50 \\
\parallel \\
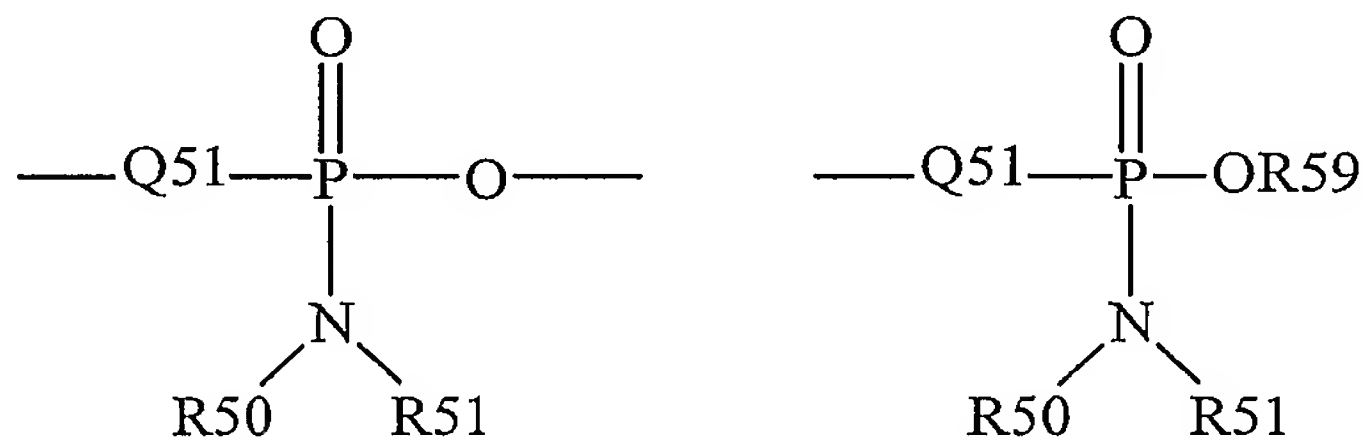-\!\!-\!\!P\!\!-\!\!- \\
\mid \\
OR59
\end{array}
$$

wherein Q50 represents S or O, and R59 represents hydrogen, a lower alkyl or an aryl. When used to substitute, e.g., an alkyl, the phosphoryl group of the phosphorylalkyl may be represented by the general formulas:

$$
\begin{array}{cc}
\begin{array}{c}
Q50 \\
\parallel \\
-Q51-\!\!P\!\!-\!\!O-\!\!- \\
\mid \\
OR59
\end{array}
&
\begin{array}{c}
Q50 \\
\parallel \\
-Q51-\!\!P\!\!-\!\!OR59 \\
\mid \\
OR59
\end{array}
\end{array}
$$

wherein Q50 and R59, each independently, are defined above, and Q51 represents O, S or N. When Q50 is S, the phosphoryl moiety is a "phosphorothioate".
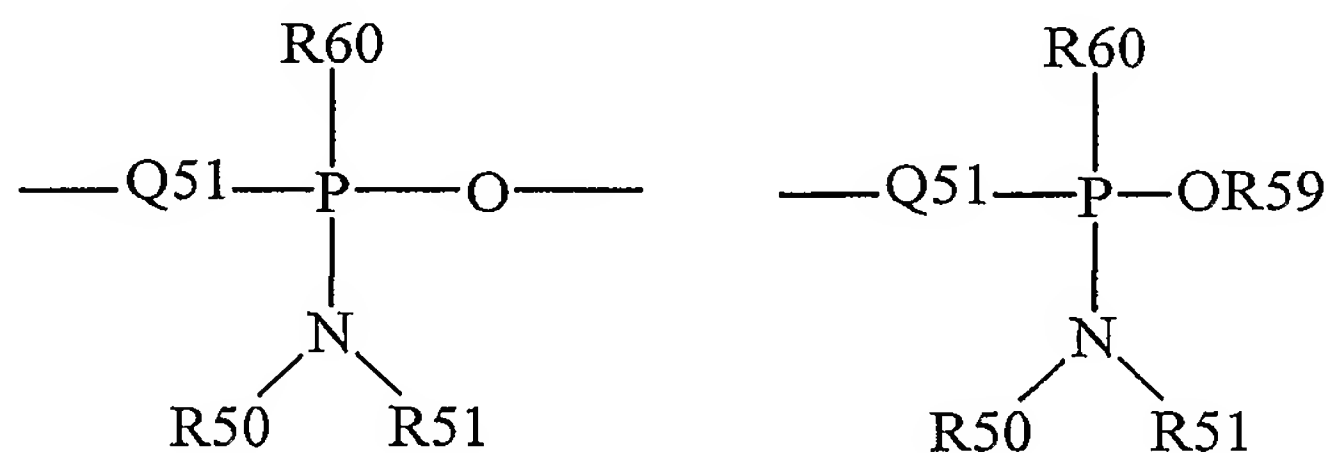
The term "phosphoramidite" is art-recognized and may be represented in the general formulas:

$$
\begin{array}{cc}
\begin{array}{c}
O \\
\parallel \\
-Q51-\!\!P\!\!-\!\!O-\!\!- \\
\mid \\
N \\
R50 \quad R51
\end{array}
&
\begin{array}{c}
O \\
\parallel \\
-Q51-\!\!P\!\!-\!\!OR59 \\
\mid \\
N \\
R50 \quad R51
\end{array}
\end{array}
$$

wherein Q51, R50, R51 and R59 are as defined above.

The term "phosphonamidite" is art-recognized and may be represented in the general formulas:

$$
\begin{array}{cc}
\begin{array}{c}
R60 \\
\mid \\
-Q51-\!\!P\!\!-\!\!O-\!\!- \\
\mid \\
N \\
R50 \quad R51
\end{array}
&
\begin{array}{c}
R60 \\
\mid \\
-Q51-\!\!P\!\!-\!\!OR59 \\
\mid \\
N \\
R50 \quad R51
\end{array}
\end{array}
$$

wherein Q51, R50, R51 and R59 are as defined above, and R60 represents a lower alkyl or an aryl.

Analogous substitutions may be made to alkenyl and alkynyl groups to produce, for example, aminoalkenyls, aminoalkynyls, amidoalkenyls, amidoalkynyls, iminoalkenyls, iminoalkynyls, thioalkenyls, thioalkynyls, carbonyl-substituted alkenyls or alkynyls.

The definition of each expression, e.g. alkyl, m, n, and the like, when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

The term "selenoalkyl" is art-recognized and refers to an alkyl group having a substituted seleno group attached thereto. Exemplary "selenoethers" which may be substituted on the alkyl are selected from one of -Se-alkyl, -Se-alkenyl, -Se-alkynyl, and -Se-$(CH_2)_m$-R61, m and R61 being defined above.

The terms triflyl, tosyl, mesyl, and nonaflyl are art-recognized and refer to trifluoromethanesulfonyl, $p$-toluenesulfonyl, methanesulfonyl, and nonafluorobutanesulfonyl groups, respectively. The terms triflate, tosylate, mesylate, and nonaflate are art-recognized and refer to trifluoromethanesulfonate ester, $p$-toluenesulfonate ester, methanesulfonate ester, and nonafluorobutanesulfonate ester functional groups and molecules that contain said groups, respectively.

The abbreviations Me, Et, Ph, Tf, Nf, Ts, and Ms represent methyl, ethyl, phenyl, trifluoromethanesulfonyl, nonafluorobutanesulfonyl, $p$-toluenesulfonyl and methanesulfonyl, respectively. A more comprhensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the Journal of Organic Chemistry; this list is typically presented in a table entitled Standard List of Abbreviations.

Certain compounds contained in compositions of the present invention may exist in particular geometric or stereoisomeric forms. In addition, polymers of the present invention may also be optically active. The present invention contemplates all such compounds, including cis- and trans-isomers, $R$- and $S$-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains

5    a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

10    It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction.
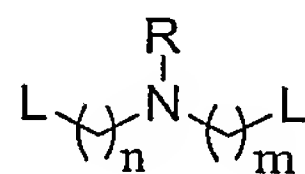
15    The term "substituted" is also contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein above. The permissible substituents may be one or more and the same or

20    different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds.

25    The phrase "protecting group" as used herein means temporary substituents which protect a potentially reactive functional group from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl ethers of alcohols, and acetals and ketals of aldehydes and ketones, respectively. The field of protecting group chemistry has been reviewed (Greene, T.W.; Wuts, P.G.M. *Protective*

30    *Groups in Organic Synthesis*, 3rd ed.; Wiley: New York, 1999). Protected forms of the inventive compounds are included within the scope of this invention.

For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, <u>Handbook of Chemistry and Physics</u>, 67th Ed., 1986-87, inside cover.

### 7. Compounds and Methods of the Invention

5    In one aspect, the present invention relates to a compound of formula **A**:

$$\text{L} \overset{R}{\underset{n}{\text{---}}} \text{N} \underset{m}{\text{---}} \text{L}$$

**A**

wherein

R is H, alkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, thioalkyl, alkenyl, alkynyl,

10 aryl, heteroaryl, aralkyl, heteroaralkyl, acyl, aminoacyl, hydroxyacyl, thioacyl, $-CO_2H$, - $(CH_2)_d-R_{80}$, or an amino acid radical;

$R_{80}$ is independently for each occurrence carboxaldehyde, carboxylate, carboxamido, alkoxycarbonyl, aryloxycarbonyl, ammonium, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocyclyl, polycyclyl, amino acid, peptide, saccharide, ribonucleic acid,

15 (deoxy)ribonucleic acid, or a ligand for a G-protein-coupled receptor;
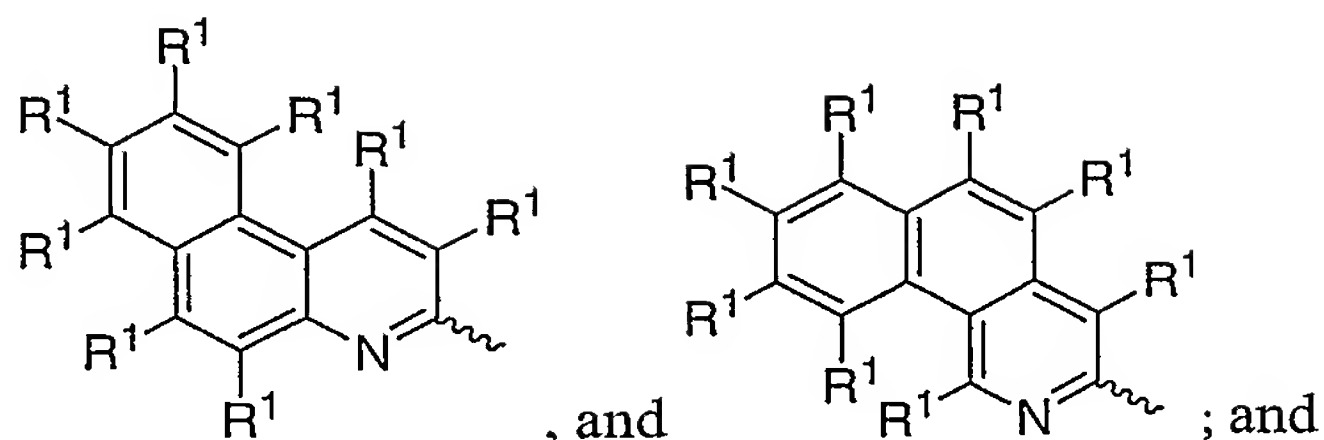
d is an integer in the range 0 to 12 inclusive;

m is an integer in the range 0 to 6 inclusive;

n is an integer in the range 0 to 6 inclusive;

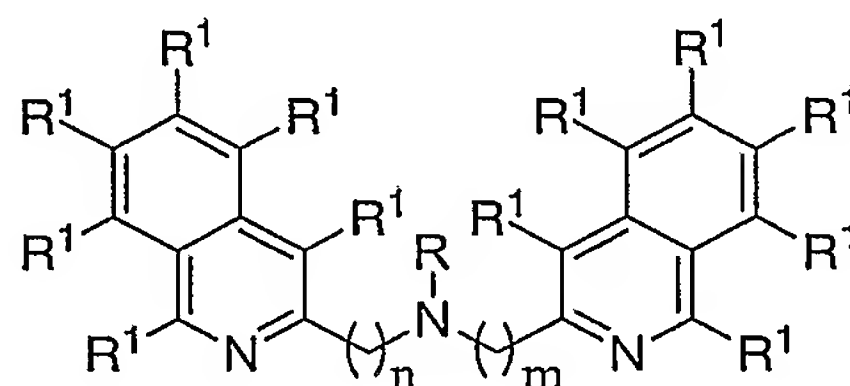L is independently for each occurrence selected from the group consisting of

20

, and  ; and

each instance of $R^1$ is selected independently from the group consisting of halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, acyl, acyloxy, acylamino, silyloxy, amino, nitro, sulfhydryl, alkylthio, imino, amido, phosphoryl, phosphonate, phosphine, carbonyl,

5    carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, cyano, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, aralkyl, heteroaralkyl, azido, aziridine, carbamoyl, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, and $-(CH_2)_d-R_{80}$.

In certain embodiments, the compounds of the present invention are represented by

10   **A** and the attendant definitions, wherein said compound is complexed with a radionuclide.

In certain embodiments, the compounds of the present invention are represented by **A** and the attendant definitions, wherein said compound is complexed with a radionuclide, wherein said radionuclide is technetium or rhenium.

In one aspect, the present invention relates to a compound of formula **B**:



15

**B**

wherein

R is H, alkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, thioalkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, acyl, aminoacyl, hydroxyacyl, thioacyl, $-CO_2H$, -

20   $(CH_2)_d-R_{80}$, or an amino acid radical;

each instance of $R^1$ is selected independently from the group consisting of halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, acyl, acyloxy, acylamino, silyloxy, amino, nitro, sulfhydryl, alkylthio, imino, amido, phosphoryl, phosphonate, phosphine, carbonyl,

- 29 -

carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, cyano, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, aralkyl, heteroaralkyl, azido, aziridine, carbamoyl, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, and $-(CH_2)_d-R_{80}$;

$R_{80}$ is independently for each occurrence carboxaldehyde, carboxylate, carboxamido, alkoxycarbonyl, aryloxycarbonyl, ammonium, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocyclyl, polycyclyl, amino acid, peptide, saccharide, ribonucleic acid, (deoxy)ribonucleic acid, or a ligand for a G-protein-coupled receptor;

d is an integer in the range 0 to 12 inclusive;

m is an integer in the range 0 to 6 inclusive; and

n is an integer in the range 0 to 6 inclusive.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein said compound is complexed with a radionuclide.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein said compound is complexed with a radionuclide, wherein said radionuclide is technetium or rhenium.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein m is 1.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein n is 1.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein m is 1; and n is 1.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein $R^1$ is hydrogen.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein m is 1; n is 1; and $R^1$ is hydrogen.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein R is $-(CH_2)_d-R_{80}$.

In certain embodiments, the compounds of the present invention are represented by

**B** and the attendant definitions, wherein m is 1; n is 1; $R^1$ is hydrogen; and R is $-(CH_2)_d-R_{80}$.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein m is 1; n is 1; $R^1$ is hydrogen; and R is $-(CH_2)_d-R_{80}$; wherein said compound is complexed with a radionuclide.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein m is 1; n is 1; $R^1$ is hydrogen; and R is $-(CH_2)_d-R_{80}$; wherein said compound is complexed with a radionuclide, wherein said radionuclide is technetium or rhenium.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein R is an amino acid radical.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein R is an amino acid radical; m is 1; and n is 1.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein R is an amino acid radical; m is 1; n is 1; and $R^1$ is hydrogen.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein R is an amino acid radical; m is 1; n is 1; $R^1$ is hydrogen; wherein said compound is complexed with a radionuclide.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein R is an amino acid radical; m is 1; n is 1; $R^1$ is hydrogen; wherein said compound is complexed with a radionuclide, wherein said radionuclide is technetium or rhenium.
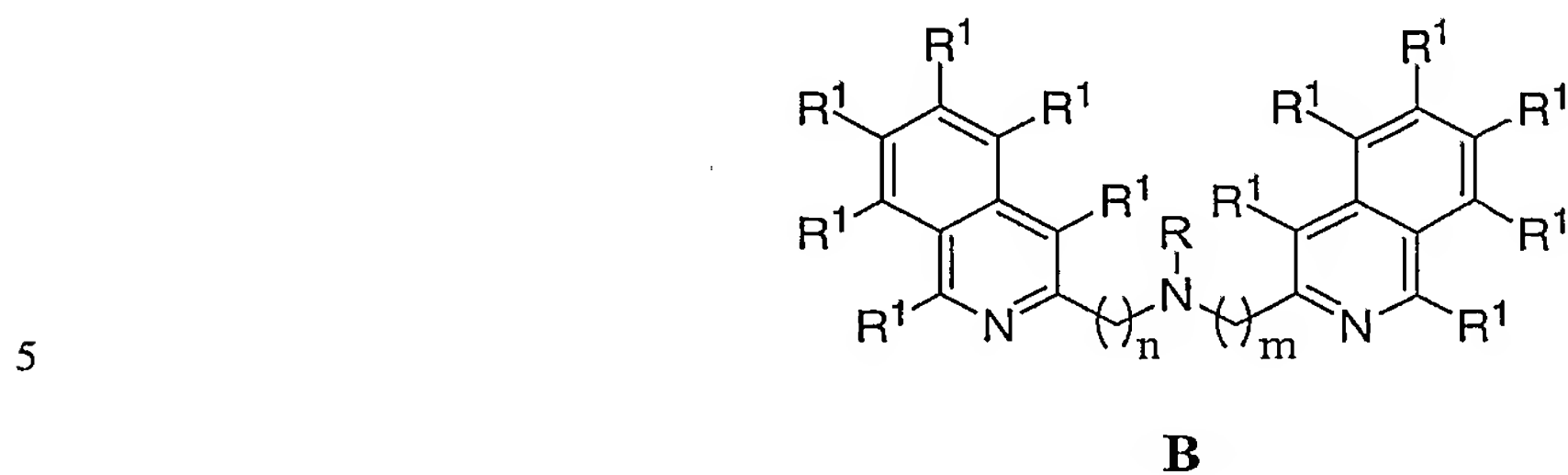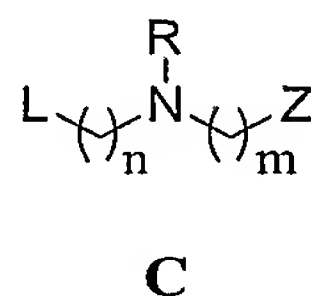
In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein the amino acid radical is $-CH_2CH_2CH_2CH_2CH(NH_2)CO_2H$.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein the amino acid radical is $-CH(CO_2H)CH_2CH_2CH_2CH_2NH_2$.

In certain embodiments, the compounds of the present invention are represented by **B** and the attendant definitions, wherein the amino acid radical is $-CH_2CH_2CO_2H$.

In certain embodiments, the compounds of the present invention are represented by
**B** and the attendant definitions, wherein the amino acid radical is
$-CH(CO_2H)(CH_2)_xCH(NH_2)CO_2H$, wherein x is an integer from 3 to 9 inclusively.

In one aspect, the present invention relates to a compound of formula **B**:



**B**

wherein R is $-CH_2CH_2CH_2CH_2CH(NH_2)CO_2H$; m is 1; n is 1; $R^1$ is hydrogen; said
compound is complexed with a radionuclide; and said radionuclide is technetium or
rhenium.

In one aspect, the present invention relates to a compound of formula **C**:



**C**

wherein

R is H, alkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, thioalkyl, alkenyl, alkynyl,
aryl, heteroaryl, aralkyl, heteroaralkyl, acyl, aminoacyl, hydroxyacyl, thioacyl, $-CO_2H$, -
$(CH_2)_d-R_{80}$, or an amino acid radical;

$R_{80}$ is independently for each occurrence carboxaldehyde, carboxylate, carboxamido,
alkoxycarbonyl, aryloxycarbonyl, ammonium, aryl, heteroaryl, cycloalkyl, cycloalkenyl,
heterocyclyl, polycyclyl, amino acid, peptide, saccharide, ribonucleic acid,
(deoxy)ribonucleic acid, or a ligand for a G-protein-coupled receptor;

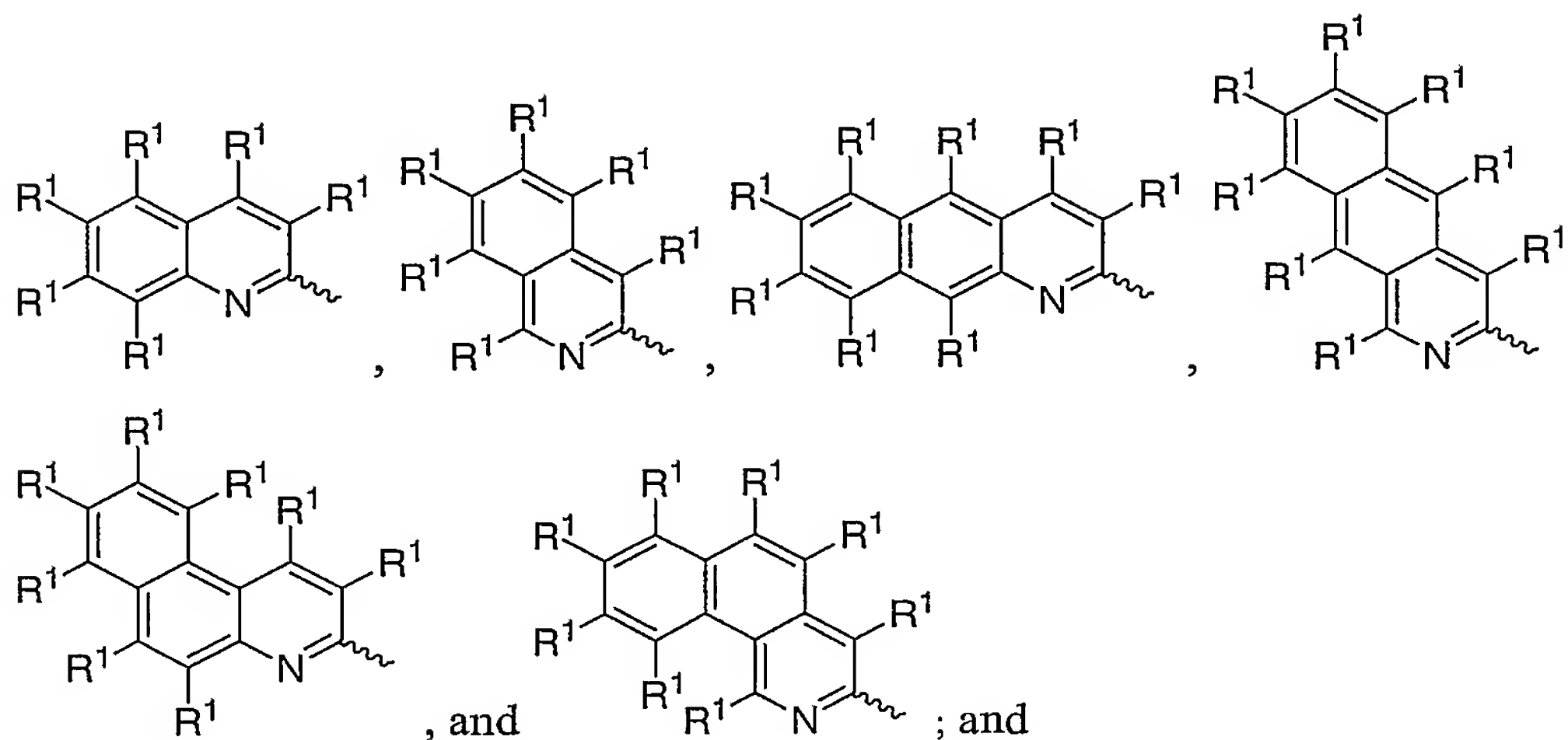d is an integer in the range 0 to 12 inclusive;

m is an integer in the range 0 to 6 inclusive;

n is an integer in the range 0 to 6 inclusive;

Z is thioalkyl, carboxylate, 2-(carboxy)aryl, 2-(carboxy)heteroaryl, 2-(hydroxy)aryl,
2-(hydroxy)heteroaryl, 2-(thiol)aryl, or 2-(thiol)heteroaryl; and
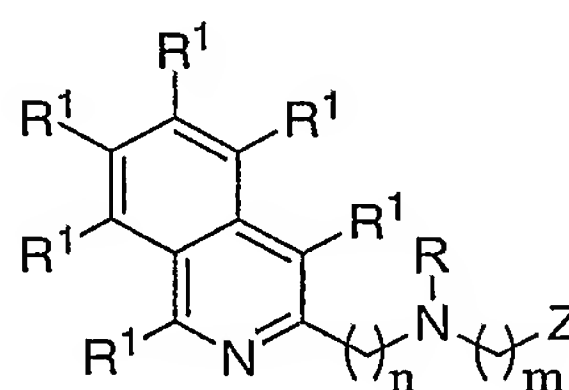
L is selected from the group consisting of



each instance of $R^1$ is selected independently from the group consisting of halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, acyl, acyloxy, acylamino, silyloxy, amino, nitro, sulfhydryl, alkylthio, imino, amido, phosphoryl, phosphonate, phosphine, carbonyl, carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, cyano, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, aralkyl, heteroaralkyl, azido, aziridine, carbamoyl, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, and $-(CH_2)_d-R_{80}$.

In certain embodiments, the compounds of the present invention are represented by **C** and the attendant definitions, wherein said compound is complexed with a radionuclide.

In certain embodiments, the compounds of the present invention are represented by **C** and the attendant definitions, wherein said compound is complexed with a radionuclide, wherein said radionuclide is technetium or rhenium.

In one aspect, the present invention relates to a compound of formula **D**:



**D**

wherein

- 33 -

R is H, alkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, thioalkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, acyl, aminoacyl, hydroxyacyl, thioacyl, -CO$_2$H, -(CH$_2$)$_d$-R$_{80}$, or an amino acid radical;

R$_{80}$ is independently for each occurrence carboxaldehyde, carboxylate, carboxamido, alkoxycarbonyl, aryloxycarbonyl, ammonium, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocyclyl, polycyclyl, amino acid, peptide, saccharide, ribonucleic acid, (deoxy)ribonucleic acid, or a ligand for a G-protein-coupled receptor;

d is an integer in the range 0 to 12 inclusive;

m is an integer in the range 0 to 6 inclusive;

n is an integer in the range 0 to 6 inclusive;

Z is thioalkyl, carboxylate, 2-(carboxy)aryl, 2-(carboxy)heteroaryl, 2-(hydroxy)aryl, 2-(hydroxy)heteroaryl, 2-(thiol)aryl, or 2-(thiol)heteroaryl; and

each instance of R$^1$ is selected independently from the group consisting of halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, acyl, acyloxy, acylamino, silyloxy, amino, nitro, sulfhydryl, alkylthio, imino, amido, phosphoryl, phosphonate, phosphine, carbonyl, carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, cyano, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, aralkyl, heteroaralkyl, azido, aziridine, carbamoyl, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, and -(CH$_2$)$_d$-R$_{80}$.

In certain embodiments, the compounds of the present invention are represented by **D** and the attendant definitions, wherein said compound is complexed with a radionuclide.

In certain embodiments, the compounds of the present invention are represented by **D** and the attendant definitions, wherein said compound is complexed with a radionuclide, wherein said radionuclide is technetium or rhenium.

In certain embodiments, the compounds of the present invention are represented by **D** and the attendant definitions, wherein Z is carboxylate.

In certain embodiments, the compounds of the present invention are represented by **D** and the attendant definitions, wherein m is 1.

In certain embodiments, the compounds of the present invention are represented by

D and the attendant definitions, wherein n is 1.

In certain embodiments, the compounds of the present invention are represented by D and the attendant definitions, wherein m is 1; and n is 1.

In certain embodiments, the compounds of the present invention are represented by D and the attendant definitions, wherein Z is carboxylate; m is 1; and n is 1.

In certain embodiments, the compounds of the present invention are represented by D and the attendant definitions, wherein $R^1$ is hydrogen.

In certain embodiments, the compounds of the present invention are represented by D and the attendant definitions, wherein Z is carboxylate; m is 1; n is 1; and $R^1$ is hydrogen.

In certain embodiments, the compounds of the present invention are represented by D and the attendant definitions, wherein R is $-(CH_2)_d-R_{80}$.

In certain embodiments, the compounds of the present invention are represented by D and the attendant definitions, wherein Z is carboxylate; m is 1; n is 1; $R^1$ is hydrogen; and R is $-(CH_2)_d-R_{80}$.

In certain embodiments, the compounds of the present invention are represented by D and the attendant definitions, wherein Z is carboxylate; m is 1; n is 1; $R^1$ is hydrogen; and R is $-(CH_2)_d-R_{80}$; wherein said compound is complexed with a radionuclide.

In certain embodiments, the compounds of the present invention are represented by D and the attendant definitions, wherein Z is carboxylate; m is 1; n is 1; $R^1$ is hydrogen; and R is $-(CH_2)_d-R_{80}$; wherein said compound is complexed with a radionuclide, wherein said radionuclide is technetium or rhenium.

In certain embodiments, the compounds of the present invention are represented by D and the attendant definitions, wherein R is an amino acid radical.

In certain embodiments, the compounds of the present invention are represented by D and the attendant definitions, wherein R is an amino acid radical; m is 1; and n is 1.

In certain embodiments, the compounds of the present invention are represented by D and the attendant definitions, wherein R is an amino acid radical; m is 1; n is 1; and $R^1$ is hydrogen.

In certain embodiments, the compounds of the present invention are represented by

**D** and the attendant definitions, wherein R is an amino acid radical; m is 1; n is 1; and $R^1$ is hydrogen; wherein said compound is complexed with a radionuclide.

In certain embodiments, the compounds of the present invention are represented by **D** and the attendant definitions, wherein R is an amino acid radical; m is 1; n is 1; and $R^1$ is hydrogen; wherein said compound is complexed with a radionuclide, wherein said radionuclide is technetium or rhenium.
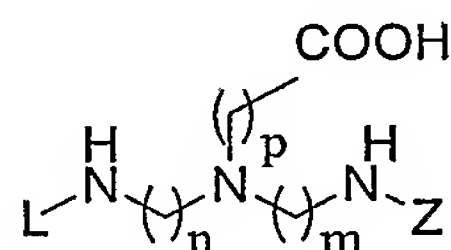
In certain embodiments, the compounds of the present invention are represented by **D** and the attendant definitions, wherein the amino acid radical is $-CH_2CH_2CH_2CH_2CH(NH_2)CO_2H$.

In certain embodiments, the compounds of the present invention are represented by **D** and the attendant definitions, wherein the amino acid radical is $-CH(CO_2H)CH_2CH_2CH_2CH_2NH_2$.

In certain embodiments, the compounds of the present invention are represented by **D** and the attendant definitions, wherein the amino acid radical is $-CH_2CH_2CO_2H$.

In certain embodiments, the compounds of the present invention are represented by **D** and the attendant definitions, wherein the amino acid radical is $-CH(CO_2H)(CH_2)_xCH(NH_2)CO_2H$, wherein x is an integer from 3 to 9 inclusively.

In one aspect, the present invention relates to a compound of formula **E**:
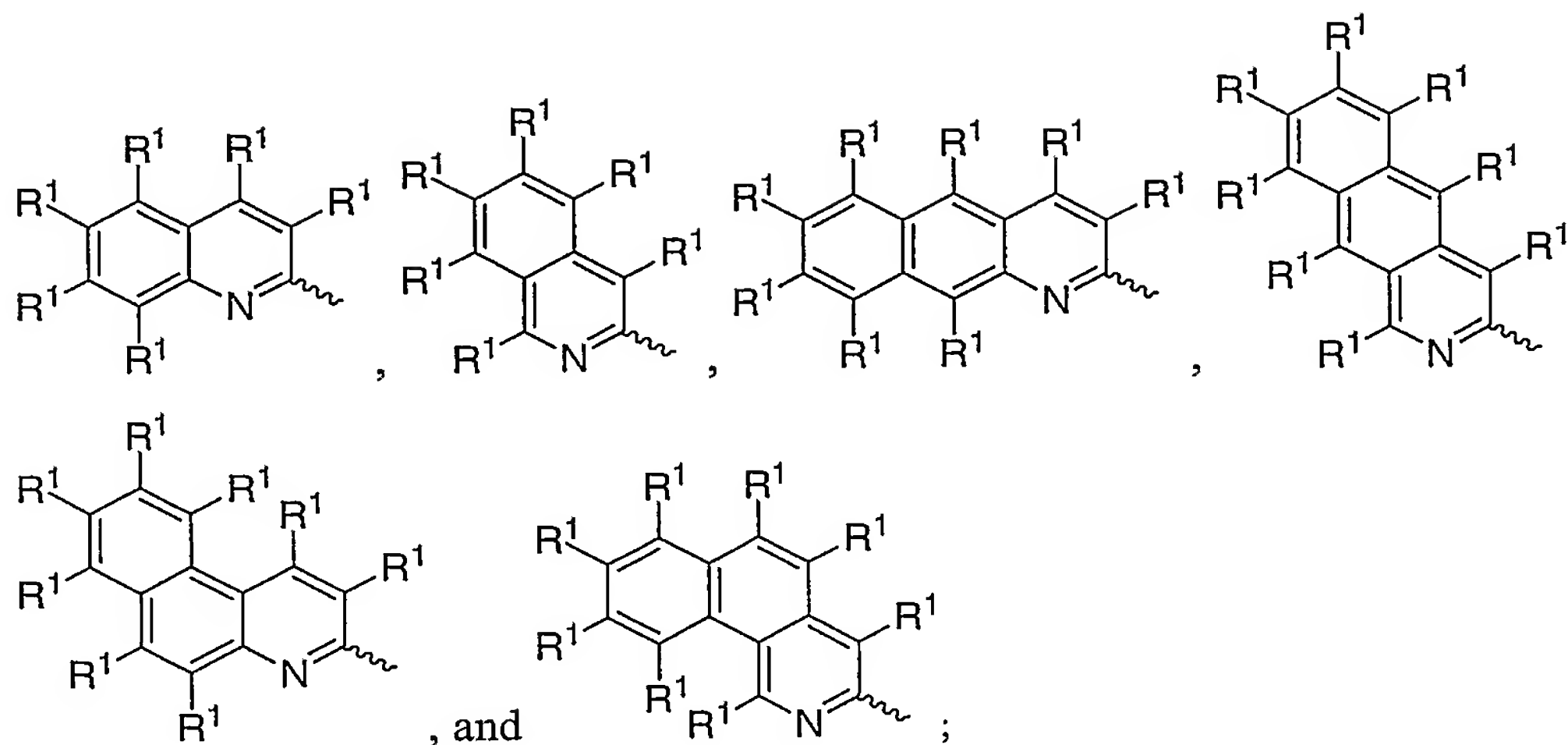


**E**

wherein

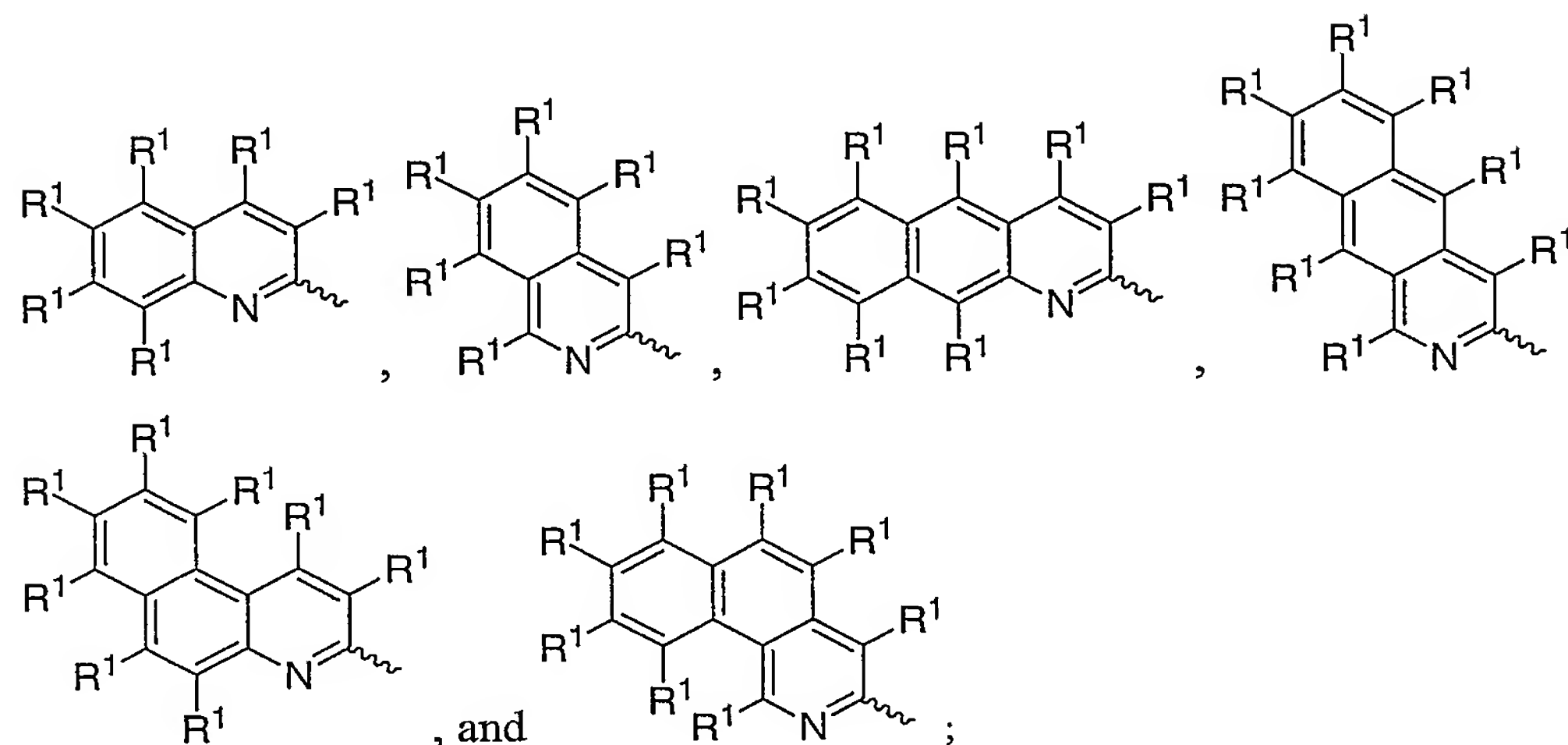m is an integer in the range 0 to 6 inclusive;

n is an integer in the range 0 to 6 inclusive;

p is an integer in the range of 1 to 10 inclusive;

Z is selected from the group consisting of $-CH_2COOH$, alkyl, aryl, aralkyl,

L is selected from the group consisting of



each instance of $R^1$ is selected independently from the group consisting of halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, acyl, acyloxy, acylamino, silyloxy, amino, nitro, sulfhydryl, alkylthio, imino, amido, phosphoryl, phosphonate, phosphine, carbonyl, carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, cyano, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, aralkyl, heteroaralkyl, azido, aziridine, carbamoyl, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, and $-(CH_2)_d-R_{80}$;

$R_{80}$ represents independently for each occurrence carboxaldehyde, carboxylate, carboxamido, alkoxycarbonyl, aryloxycarbonyl, ammonium, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocyclyl, polycyclyl, amino acid, peptide, saccharide, ribonucleic acid, (deoxy)ribonucleic acid, or a ligand for a G-protein-coupled receptor; and
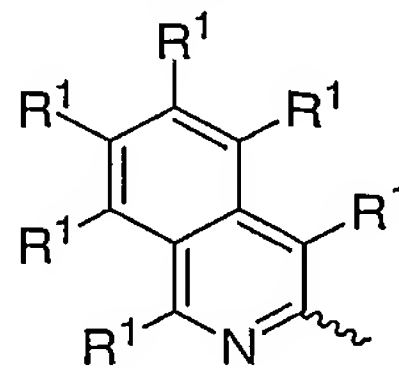
d is an integer in the range 0 to 12 inclusive.

In certain embodiments, the compounds of the present invention are represented by **E** and the attendant definitions, wherein said compound is complexed with a radionuclide.
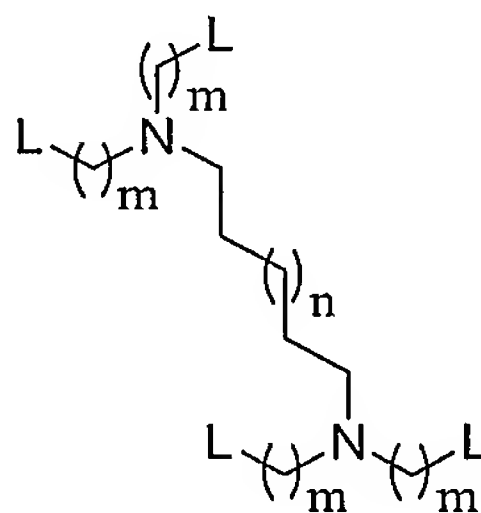
In certain embodiments, the compounds of the present invention are represented by **E** and the attendant definitions, wherein said compound is complexed with a radionuclide, wherein said radionuclide is technetium or rhenium.

In certain embodiments, the compounds of the present invention are represented by

**E** and the attendant definitions, wherein L is [structure] ; $R^1$ is hydrogen; and Z is alkyl.

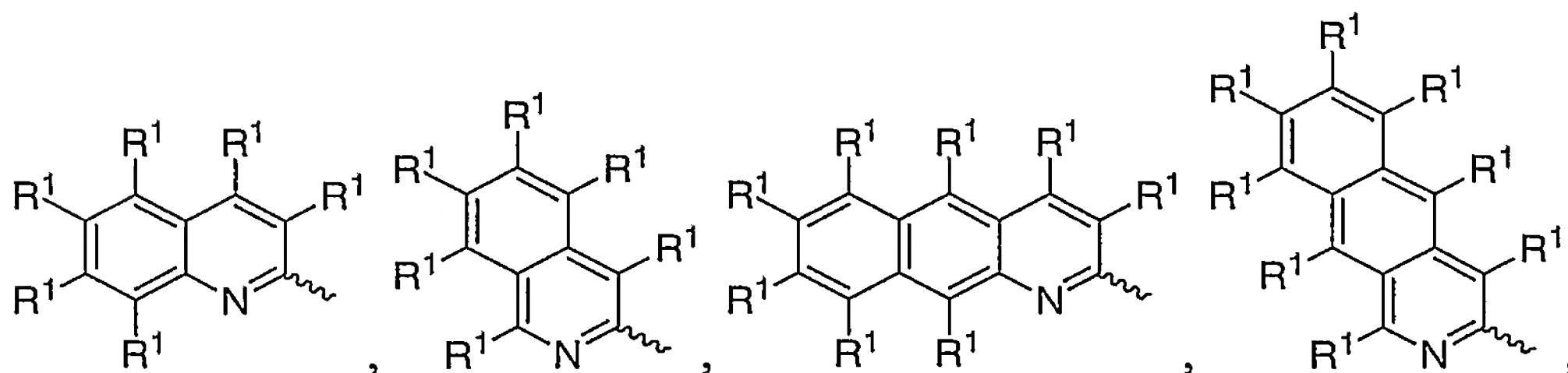In one aspect, the present invention relates to a compound of formula **F**:
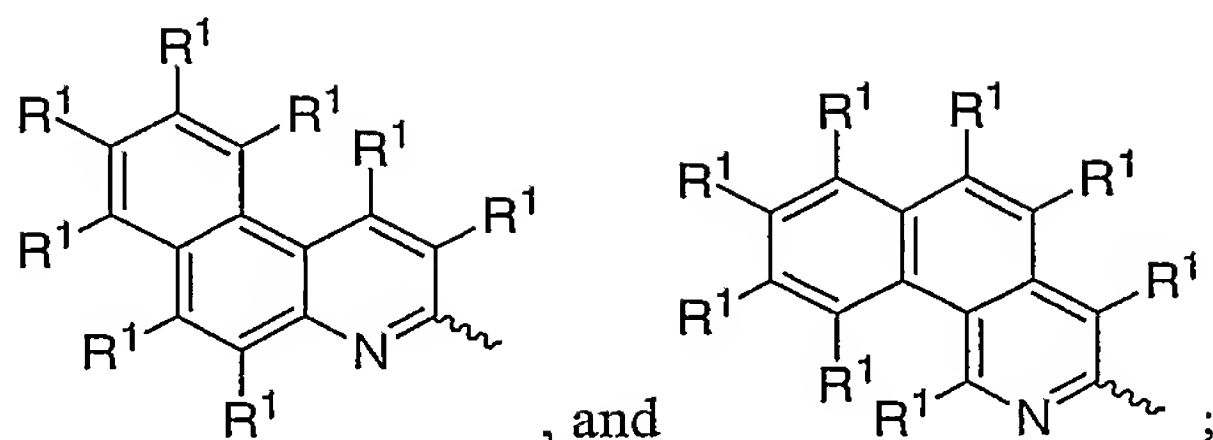


**F**

wherein

m is independently for each occurrence an integer in the range 0 to 6 inclusive;

n is an integer in the range 0 to 6 inclusive;

L is independently for each occurrence selected from the group consisting of

, and



;

each instance of $R^1$ is selected independently from the group consisting of halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, acyl, acyloxy, acylamino, silyloxy, amino, nitro, sulfhydryl, alkylthio, imino, amido, phosphoryl, phosphonate, phosphine, carbonyl,

5    carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, cyano, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, aralkyl, heteroaralkyl, azido, aziridine, carbamoyl, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, and $-(CH_2)_d-R_{80}$;
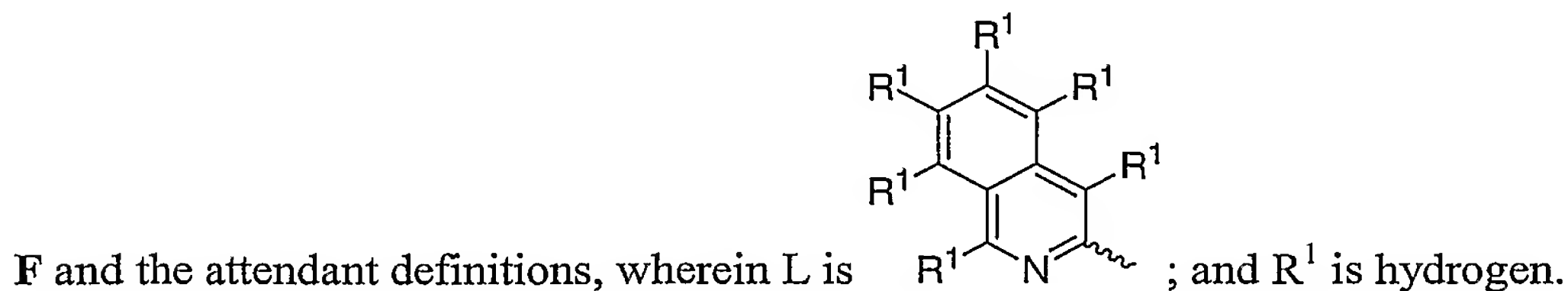
$R_{80}$ is independently for each occurrence carboxaldehyde, carboxylate, carboxamido,

10    alkoxycarbonyl, aryloxycarbonyl, ammonium, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocyclyl, polycyclyl, amino acid, peptide, saccharide, ribonucleic acid, (deoxy)ribonucleic acid, or a ligand for a G-protein-coupled receptor; and

d is an integer in the range 0 to 12 inclusive.

In certain embodiments, the compounds of the present invention are represented by

15    **F** and the attendant definitions, wherein m is 1.

In certain embodiments, the compounds of the present invention are represented by **F** and the attendant definitions, wherein n is 1.

In certain embodiments, the compounds of the present invention are represented by

**F** and the attendant definitions, wherein L is      ; and $R^1$ is hydrogen.

20    In certain embodiments, the compounds of the present invention are represented by

**F** and the attendant definitions, wherein L is      ; $R^1$ is hydrogen, m is 1; and n

is 1.

In certain embodiments, the compounds of the present invention are represented by F and the attendant definitions, wherein said compound is complexed with a radionuclide.

In certain embodiments, the compounds of the present invention are represented by F and the attendant definitions, wherein said compound is complexed with a radionuclide, wherein said radionuclide is technetium or rhenium.

In one aspect, the present invention relates to a compound of formula G:



**G**

wherein

R is H, alkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, thioalkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, acyl, aminoacyl, hydroxyacyl, thioacyl, $-CO_2H$, - $(CH_2)_d$-$R_{80}$, or an amino acid radical;

$R_{80}$ is independently for each occurrence carboxaldehyde, carboxylate, carboxamido, alkoxycarbonyl, aryloxycarbonyl, ammonium, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocyclyl, polycyclyl, amino acid, peptide, saccharide, ribonucleic acid, (deoxy)ribonucleic acid, or a ligand for a G-protein-coupled receptor;
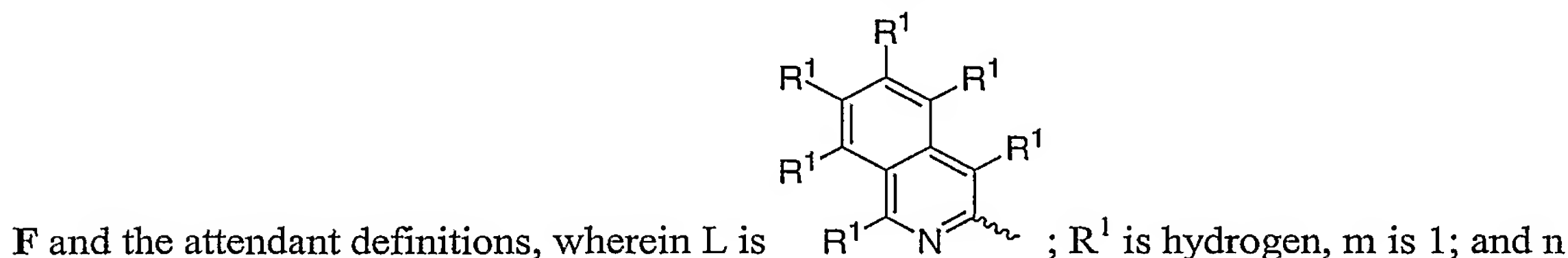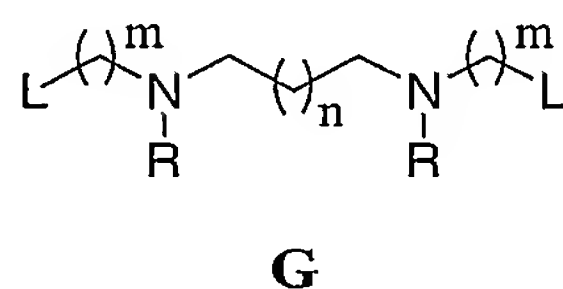
d is an integer in the range 0 to 12 inclusive;

m is independently for each occurrence an integer in the range 0 to 6 inclusive;

n is an integer in the range 0 to 6 inclusive;

L is independently for each occurrence selected from the group consisting of

, and



; and

each instance of $R^1$ is selected independently from the group consisting of halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, acyl, acyloxy, acylamino, silyloxy, amino, nitro, sulfhydryl, alkylthio, imino, amido, phosphoryl, phosphonate, phosphine, carbonyl,

5    carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, cyano, guanidine, amidine, acetal, ketal, amine oxide, aryl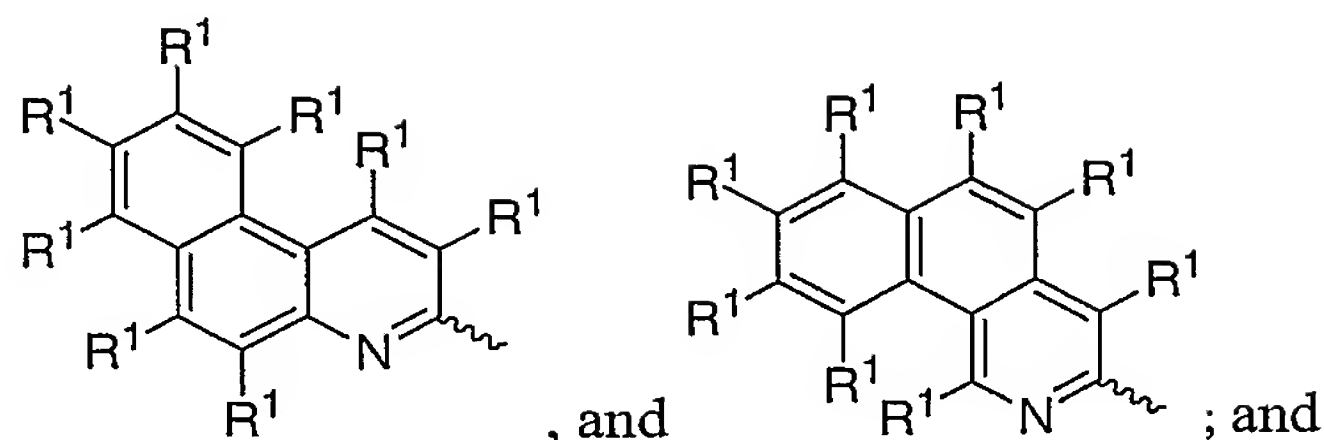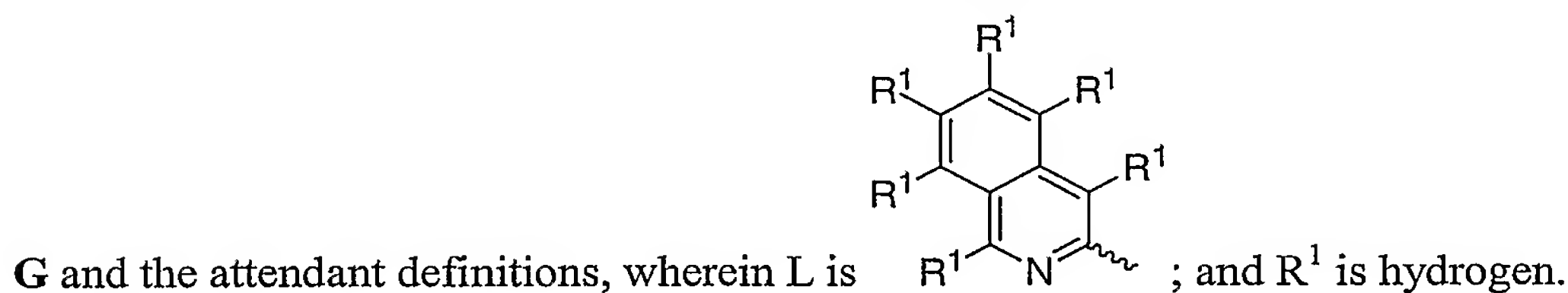, heteroaryl, aralkyl, heteroaralkyl, azido, aziridine, carbamoyl, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, and $-(CH_2)_d-R_{80}$.

In certain embodiments, the compounds of the present invention are represented by

10   $\mathbf{G}$ and the attendant definitions, wherein m is 1.

In certain embodiments, the compounds of the present invention are represented by $\mathbf{G}$ and the attendant definitions, wherein n is 1.

In certain embodiments, the compounds of the present invention are represented by $\mathbf{G}$ and the attendant definitions, wherein R is hydrogen.

15   In certain embodiments, the compounds of the present invention are represented by

$\mathbf{G}$ and the attendant definitions, wherein L is  ; and $R^1$ is hydrogen.

In certain embodiments, the compounds of the present invention are represented by

$\mathbf{G}$ and the attendant definitions, wherein L is  ; $R^1$ is hydrogen; m is 1; n is 1; and R is hydrogen.

20   In certain embodiments, the compounds of the present invention are represented by $\mathbf{G}$ and the attendant definitions, wherein said compound is complexed with a radionuclide.
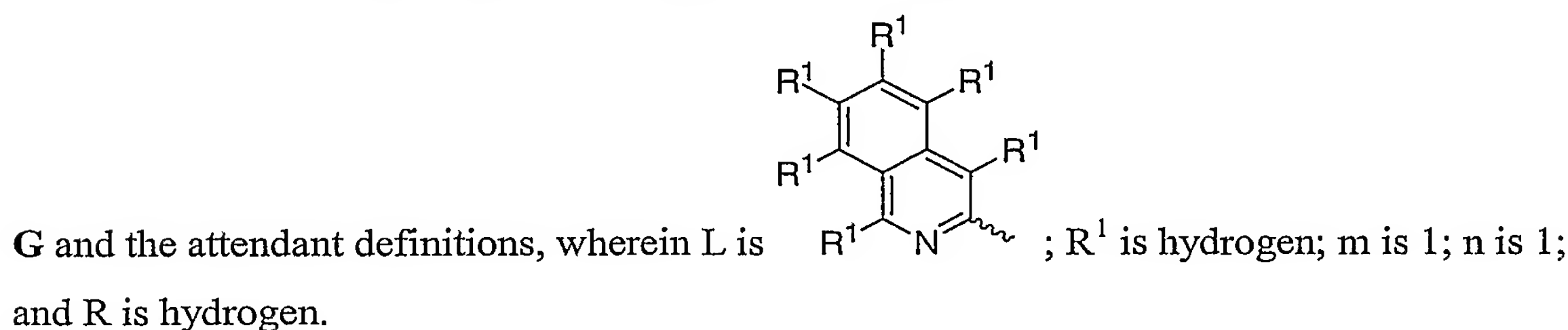
In certain embodiments, the compounds of the present invention are represented by G and the attendant definitions, wherein said radionuclide is technetium or rhenium.

In one aspect, the present invention relates to a compound of formula **H**:

$$L \text{-} (\phantom{})_m \overset{|}{\underset{R^2}{N}} \text{-} (\phantom{})_n \overset{|}{\underset{R}{N}} \text{-} (\phantom{})_n \overset{|}{\underset{R^2}{N}} (\phantom{})_m \text{-} L$$

**H**

wherein

R is hydrogen, halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, acyl, acyloxy, acylamino, silyloxy, amino, nitro, sulfhydryl, alkylthio, imino, amido, phosphoryl, phosphonate, phosphine, carbonyl, carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, cyano, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, aralkyl, heteroaralkyl, azido, aziridine, carbamoyl, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, or $-(CH_2)_d\text{-}R_{80}$;

$R^2$ represents a moiety comprising a neutral or anionic Lewis base, H, alkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, thioalkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, acyl, aminoacyl, hydroxyacyl, thioacyl, (amino)alkoxycarbonyl, (hydroxy)alkoxycarbonyl, (amino)alkylaminocarbonyl, (hydroxy)alkylaminocarbonyl, $-CO_2H$, $-(CH_2)_d\text{-}R_{80}$, or an amino acid radical;

$R_{80}$ is independently for each occurrence carboxaldehyde, carboxylate, carboxamido, alkoxycarbonyl, aryloxycarbonyl, ammonium, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocyclyl, polycyclyl, amino acid, peptide, saccharide, ribonucleic acid, (deoxy)ribonucleic acid, or a ligand for a G-protein-coupled receptor;

d is an integer in the range 0 to 12 inclusive;

m is an integer in the range 0 to 6 inclusive;

n is an integer in the range 0 to 6 inclusive;

L is independently for each occurrence selected from the group consisting of

, and                    ; and

each instance of $R^1$ is selected independently from the group consisting of halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, acyl, acyloxy, acylamino, silyloxy, amino, nitro, sulfhydryl, alkylthio, imino, amido, phosphoryl, phosphonate, phosphine, carbonyl, carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, cyano, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, aralkyl, heteroaralkyl, azido, aziridine, carbamoyl, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, and $-(CH_2)_d-R_{80}$.

In certain embodiments, the compounds of the present invention are represented by **H** and the attendant definitions, wherein m is 1.

In certain embodiments, the compounds of the present invention are represented by **H** and the attendant definitions, wherein n is 1.

In certain embodiments, the compounds of the present invention are represented by **H** and the attendant definitions, wherein R is hydrogen or $-(CH_2)_d-R_{80}$.

In certain embodiments, the compounds of the present invention are represented by **H** and the attendant definitions, wherein $R^2$ is a moiety comprising an anionic Lewis base

In certain embodiments, the compounds of the present invention are represented by **H** and the attendant definitions, wherein $R^2$ is a carboxylate, thiolate, or phenolate

In certain embodiments, the compounds of the present invention are represented by

H and the attendant definitions, wherein L is [structure]; and $R^1$ is hydrogen.

In certain embodiments, the compounds of the present invention are represented by

H and the attendant definitions, wherein L is [structure]; $R^1$ is hydrogen; m is 1; n is 1; R is hydrogen or $-(CH_2)_d-R_{80}$; and $R^2$ is a carboxylate, thiolate, or phenolate.

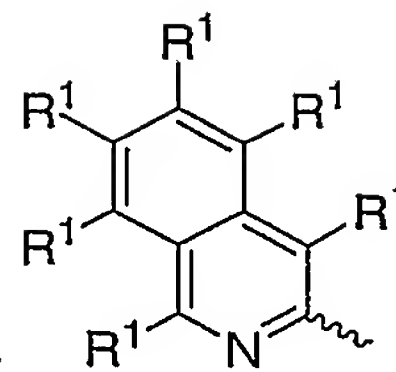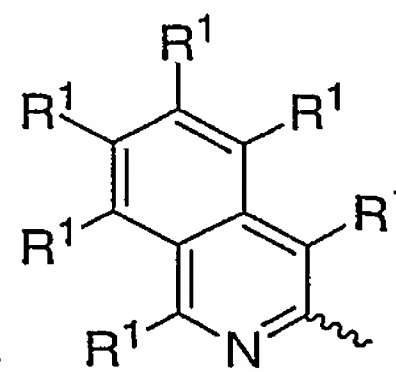In certain embodiments, the compounds of the present invention are represented by H and the attendant definitions, wherein said compound is complexed with a radionuclide.

In certain embodiments, the compounds of the present invention are represented by H and the attendant definitions, wherein said compound is complexed with a radionuclide, wherein said radionuclide is technetium or rhenium.

In certain embodiments, the present invention relates to a formulation, comprising a compound represented by A to H and the attendant definitions; and a pharmaceutically acceptable excipient.

In certain embodiments, the present invention relates to a method of imaging a region in a patient, comprising administering to the patient a diagnostically effective amount of a compound represented by A to H, wherein the compound is complexed with a radionuclide.

In certain embodiments, the present invention relates to a method of imaging a region in a patient, wherein said region of said patient is the head or thorax, comprising administering to the patient a diagnostically effective amount of a compound represented by A to H, wherein the compound is complexed with a radionuclide.

In certain embodiments, the present invention relates to a method of preparing a peptide conjugate incorporating a compound of claim represented by A to H, wherein the peptide conjugate is prepared using solid phase synthetic techniques.

The novel ligands described above, may be incorporated into radionuclide

complexes used as radiographic imaging agents.  Further, these ligands or complexes can be covalently or non-covalently attached to biologically active carrier molecules, such as, antibodies, enzymes, peptides peptidomimetics, hormones, and the like.  The complexes of the present invention are prepared by reacting one of the aforementioned ligands with a

5    radionuclide containing solution under radionuclide complex forming reaction conditions.  In particular, if a technetium agent is desired, the reaction is carried out with a pertechnetate solution under technetium-99m complex forming reaction conditions.  The solvent may then be removed by any appropriate means, such as evaporation.  The complexes are then prepared for administration to the patient by dissolution or suspension in a pharmaceutically

10   acceptable vehicle.

The present invention also relates to imaging agents containing a radionuclide complex as described above, in an amount sufficient for imaging, together with a pharmaceutically acceptable radiological vehicle. The radiological vehicle should be suitable for injection or aspiration, such as human serum albumin; aqueous buffer solutions,

15   e.g., tris(hydromethyl) aminomethane (and its salts), phosphate, citrate, bicarbonate, etc; sterile water; physiological saline; and balanced ionic solutions containing chloride and or dicarbonate salts or normal blood plasma cations such as calcium, potassium, sodium, and magnesium.

The concentration of the imaging agent according to the present invention in the

20   radiological vehicle should be sufficient to provide satisfactory imaging, for example, when using an aqueous solution, the dosage is about 1.0 to 50 millicuries. The imaging agent should be administered so as to remain in the patient for about 1 to 3 hours, although both longer and shorter time periods are acceptable. Therefore, convenient ampules containing 1 to 10 mL of aqueous solution may be prepared.

25   Imaging may be carried out in the normal manner, for example by injecting a sufficient amount of the imaging composition to provide adequate imaging and then scanning with a suitable machine, such as a gamma camera.  In certain embodiments, the present invention relates to a method of imaging a region in a patient, comprising the steps of:  administering to a patient a diagnostically effective amount of a compound of the

30   present invention complexed with a radionuclide; exposing a region of said patient to radiation; and obtaining an image of said region of said patient.  In certain embodiments of the method of imaging a region in a patient, said region of said patient is the head or thorax.

*8. Pharmaceutical Formulations*

In another aspect, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of one or more of the compounds described above, formulated together with one or more pharmaceutically

5      acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses,

10     powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; (3) topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually;

15     (6) ocularly; (7) transdermally; or (8) nasally.

The phrase "therapeutically-effective amount" as used herein means that amount of a compound, material, or composition comprising a compound of the present invention which is effective for producing some desired therapeutic effect in at least a sub-population of cells in an animal at a reasonable benefit/risk ratio applicable to any medical treatment.

20     The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

25     The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible

30     with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1)

sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato
starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl
cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8)
excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil,

5      cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols,
such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene
glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents,
such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-
free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered

10     solutions; (21) polyesters, polycarbonates and/or polyanhydrides; and (22) other non-toxic
compatible substances employed in pharmaceutical formulations.

Formulations of the present invention may be based in part on liposomes.
Liposomes consist of a phospholipid bilayer which forms a shell around an aqueous core.
Methods for preparing liposomes for administration to a patient are known to those skilled

15     in the art; for example, U.S. Pat. No. 4,798,734 describes methods for encapsulation of
biological materials in liposomes. The biological material is dissolved in a aqueous
solution, and the appropriate phospholipids and lipids are added, along with surfactants if
required. The material is then dialyzed or sonicated, as necessary. A review of known
methods is presented by G. Gregoriadis, Chapter 14 ("Liposomes"), in Drug Carriers in

20     Biology and Medicine, pp. 287-341 (Academic Press, 1979).

Formulations of the present invention may be based in part on polymeric
microparticles. Microspheres formed of polymers or proteins are also well known to those
skilled in the art, and can be tailored for passage through the gastrointestinal tract, as
described in U.S. Pat. Nos. 4,906,474, 4,925,673, and 3,625,214, for example. There are a

25     number of well-known methods, including solvent evaporation and coacervation/phase
separation, for preparing microspheres. Bioerodible microspheres can be prepared using
any of the methods developed for making microspheres for drug delivery, as described, for
example, by Mathiowitz et al., J. Appl. Polymer Sci. 35, 755-774(1988), and P. Deasy, in
Microencapsulation and Related Drug Processes, pp. 61-193, (Dekker, 1984), the teachings

30     of which are incorporated herein. The selection of a method depends on the drug properties
and choice of polymer, as well as the size, external morphology, and degree of crystallinity
desired, as discussed, for example, by Benita et al., J. Pharm. Sci. 73, 1721-1724 (1984),

Jalil and Nixon, J. Microencapsulation, 7, 297-325(1990), and Mathiowitz et al., Scanning Microscopy 4, 329-340(1990), the teachings of which are incorporated herein.

In solvent evaporation, described, for example, in Mathiowitz et al., (1990), Benita, and U.S. Pat. No. 4,272,398 to Jaffe, the polymer is dissolved in a volatile organic solvent.

5    The drug, either in soluble or particulate form, is added to the polymer solution and the mixture is suspended in an aqueous phase containing a surface active agent such as poly(vinyl alcohol). The resulting emulsion is stirred until most of the organic solvent evaporates, leaving solid microspheres. Microspheres of various sizes (1-1000 microns) and morphologies may be obtained by this method, which is useful for non-labile polymers.

10    Coacervation/phase separation techniques have been used to encapsulate both solid and liquid core materials with various polymer coatings. U.S. Pat. Nos. 2,730,456, 2,730,457, and 2,800,457 to Green and Schleichter, describe gelatin and gelatin-acacia (gum arabic) coating systems, for example. Simple coacervation employs a single colloid (e.g. gelatin in water) and involves the removal of the associated water from around the

15    dispersed colloid by agents with a higher affinity for water, such as alcohols and salts. Complex coacervation employs more than one colloid, and the separation proceeds mainly by charge neutralization of the colloids carrying opposite charges rather than by dehydration. Coacervation may also be induced using nonaqueous vehicles, as described in Nakano et al., Int. J. Pharm, 4, 29-298 (1980), for example.

20    Hydrogel microspheres made of gel-type polymers such as alginate or polyphosphazenes or other dicarboxylic polymers can be prepared by dissolving the polymer in an aqueous solution, suspending the material to be incorporated into the mixture, and extruding the polymer mixture through a microdroplet forming device, equipped with a nitrogen gas jet. The resulting microspheres fall into a slowly stirring, ionic

25    hardening bath, as illustrated, for example, by Salib, et al., Pharmazeutische Industrie 40-11A, 1230 (1978), the teachings of which are incorporated herein. The advantage of this system is the ability to further modify the surface of the microspheres by coating them with polycationic polymers (such as polylysine) after fabrication, as described, for example, by Lim et al, J. Pharm Sci. 70, 351-354 (1981). The microsphere particle size depends upon the

30    extruder size as well as the polymer and gas flow rates.

Examples of polymers that can be used include polyamides, polycarbonates,

polyalkylenes and derivatives thereof including, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polymers of acrylic and methacrylic esters, including poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl

5      methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate), polyvinyl polymers including polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, poly(vinyl acetate), and polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, celluloses including alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers,

10     cellulose esters, nitro celluloses, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, and cellulose sulphate sodium salt, polypropylene, polyethylenes including poly(ethylene glycol), poly(ethylene oxide), and poly(ethylene terephthalate), and

15     polystyrene.

Examples of biodegradable polymers include synthetic polymers such as polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, polyurethanes, poly(butic acid), poly(valeric acid), and poly(lactide-cocaprolactone), and natural polymers such as alginate and other polysaccharides including dextran and cellulose, collagen, chemical

20     derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water in vivo, by surface or bulk erosion.

25     Bioadhesive polymers of particular interest include bioerodible hydrogels described by H. S. Sawhney, C. P. Pathak and J. A. Hubbell in Macromolecules, 1993, 26, 581-587, the teachings of which are incorporated herein, polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate),

30     poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

A diluent used in a composition of the present invention can be one or more compounds which are capable of densifying the active principle to give the desired mass. The preferred diluents are mineral phosphates such as calcium phosphates; sugars such as hydrated or anhydrous lactose, or mannitol; and cellulose or cellulose derivatives, for example microcrystalline cellulose, starch, corn starch or pregelatinized starch. Very particularly preferred diluents are lactose monohydrate, mannitol, microcrystalline cellulose and corn starch, used by themselves or in a mixture, for example a mixture of lactose monohydrate and corn starch or a mixture of lactose monohydrate, corn starch and microcrystalline cellulose.

A binder employed in a composition of the present invention can be one or more compounds which are capable of densifying a compound of formula (I), converting it to coarser and denser particles with better flow properties. The preferred binders are alginic acid or sodium alginate; cellulose and cellulose derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose or methyl cellulose, gelatin; acrylic acid polymers; and povidone, for example povidone K-30; hydroxypropyl methyl cellulose and povidone K-30 are very particularly preferred binders.

A disintegrating agent employed in a composition of the present invention can be one or more compounds which facilitate the disintegration of the prepared formulation when it is placed in an aqueous medium. The preferred disintegrating agents are cellulose or cellulose derivatives such as sodium carboxymethyl cellulose, crosslinked sodium carboxymethyl cellulose, micro-crystalline cellulose, cellulose powder, crospovidone; pregelatinized starch, sodium starch glyconate, sodium carboxymethyl starch, or starch. Crospovidone, crosslinked sodium carboxymethyl cellulose and sodium carboxymethyl starch are preferred disintegrating agents.

An antiadhesive employed in a composition of the present invention can be one or more compounds which are capable of reducing the sticky character of the formulation, for example of preventing adhesion to metal surfaces. The preferred antiadhesives are compounds containing silicon, for example silica or talcum.

A flow promoter employed in a composition of the present invention can be one or more compounds which are capable of facilitating the flow of the prepared formulation. The

preferred flow promoters are compounds containing silicon, for example anhydrous colloidal silica or precipitated silica.

A lubricant employed in a composition of the present invention can be one or more compounds which are capable of preventing the problems associated with the preparation of dry forms, such as the sticking and/or seizing problems which occur in the machines during compression or filling. The preferred lubricants are fatty acids or fatty acid derivatives such as calcium stearate, glyceryl monostearate, glyceryl palmitostearate, magnesium stearate, sodium laurylsulfate, sodium stearylfumarate, zinc stearate or stearic acid; hydrogenated vegetable oils, for example hydrogenated castor oil; polyalkylene glycols or polyethylene glycol; sodium benzoate; or talcum. Magnesium stearate or sodium stearylfumarate is preferred according to the present invention.

A color employed in a formulation of the present invention can be one or more compounds which are capable of imparting the desired color to the prepared formulation. The addition of a color can serve for example to differentiate between formulations containing different doses of active principle. The preferred colors are iron oxides.

As set out above, certain embodiments of the present compounds may contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable acids. The term "pharmaceutically-acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed during subsequent purification. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19)

The pharmaceutically acceptable salts of the subject compounds include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts

include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicyclic, sulfanilic, 2-

5      acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

In other cases, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable bases. The term "pharmaceutically-acceptable salts" in

10     these instances refers to the relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, with ammonia, or

15     with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. (See, for example, Berge et al.,

20     *supra*)

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

25     Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric

30     acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

In certain embodiments, a formulation of the present invention comprises an excipient selected from the group consisting of cyclodextrins, liposomes, micelle forming agents, e.g., bile acids, and polymeric carriers, e.g., polyesters and polyanhydrides; and a compound of the present invention. In certain embodiments, an aforementioned formulation renders orally bioavailable a compound of the present invention.

Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets,

pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example,

5      carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol, glycerol

10     monostearate, and non-ionic surfactants; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and

15     hard-shelled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant

20     (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or

25     prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be formulated for rapid release, e.g.,

30     freeze-dried. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately

before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations

containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a

5 pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones,

10 bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted

15 hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can

20 be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration

25 comprise one or more compounds of the invention in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain sugars, alcohols, antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the

30 blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in

the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms upon the subject compounds may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given in forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administrations are preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticulare, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

These compounds may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide

thereof, the route of administration, the time of administration, the rate of excretion or metabolism of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In general, a suitable daily dose of a compound of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, intravenous, intracerebroventricular and subcutaneous doses of the compounds of this invention for a patient, when used for the indicated analgesic effects, will range from about 0.0001 to about 100 mg per kilogram of body weight per day.

If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition).

In another aspect, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of one or more of the subject compounds, as described above, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes for application to the tongue; (2)

parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin, lungs, or oral cavity; or (4) intravaginally or intravectally, for example, as a pessary, cream or foam; (5) sublingually; (6) ocularly; (7) transdermally; or (8) nasally.

The compounds according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other pharmaceuticals.

The term "treatment" is intended to encompass also prophylaxis, therapy and cure.

The patient receiving this treatment is any animal in need, including primates, in particular humans, and other mammals such as equines, cattle, swine and sheep; and poultry and pets in general.

The compound of the invention can be administered as such or in admixtures with pharmaceutically acceptable carriers and can also be administered in conjunction with antimicrobial agents such as penicillins, cephalosporins, aminoglycosides and glycopeptides. Conjunctive therapy, thus includes sequential, simultaneous and separate administration of the active compound in a way that the therapeutical effects of the first administered one is not entirely disappeared when the subsequent is administered.

*9. Combinatorial Libraries*

The subject compounds readily lend themselves to the creation of combinatorial libraries for the screening of pharmaceutical, agrochemical or other biological or medically-related activity or material-related qualities. A combinatorial library for the purposes of the present invention is a mixture of chemically related compounds which may be screened together for a desired property; said libraries may be in solution or covalently linked to a solid support. The preparation of many related compounds in a single reaction greatly reduces and simplifies the number of screening processes which need to be carried out. Screening for the appropriate biological, pharmaceutical, agrochemical or physical property may be done by conventional methods.

Diversity in a library can be created at a variety of different levels. For instance, the substrate aryl groups used in a combinatorial approach can be diverse in terms of the core

aryl moiety, e.g., a variegation in terms of the ring structure, and/or can be varied with respect to the other substituents.

A variety of techniques are available in the art for generating combinatorial libraries of small organic molecules. See, for example, Blondelle et al. (1995) Trends Anal. Chem. 14:83; the Affymax U.S. Patents 5,359,115 and 5,362,899: the Ellman U.S. Patent 5,288,514: the Still et al. PCT publication WO 94/08051; Chen et al. (1994) JACS 116:2661: Kerr et al. (1993) JACS 115:252; PCT publications WO92/10092, WO93/09668 and WO91/07087; and the Lerner et al. PCT publication WO93/20242). Accordingly, a variety of libraries on the order of about 16 to 1,000,000 or more diversomers can be synthesized and screened for a particular activity or property.

In an exemplary embodiment, a library of substituted diversomers can be synthesized using the subject reactions adapted to the techniques described in the Still et al. PCT publication WO 94/08051, e.g., being linked to a polymer bead by a hydrolyzable or photolyzable group, e.g., located at one of the positions of substrate. According to the Still et al. technique, the library is synthesized on a set of beads, each bead including a set of tags identifying the particular diversomer on that bead. In one embodiment, which is particularly suitable for discovering enzyme inhibitors, the beads can be dispersed on the surface of a permeable membrane, and the diversomers released from the beads by lysis of the bead linker. The diversomer from each bead will diffuse across the membrane to an assay zone, where it will interact with an enzyme assay. Detailed descriptions of a number of combinatorial methodologies are provided below.

*A) Direct Characterization*

A growing trend in the field of combinatorial chemistry is to exploit the sensitivity of techniques such as mass spectrometry (MS), *e.g.*, which can be used to characterize sub-femtomolar amounts of a compound, and to directly determine the chemical constitution of a compound selected from a combinatorial library. For instance, where the library is provided on an insoluble support matrix, discrete populations of compounds can be first released from the support and characterized by MS. In other embodiments, as part of the MS sample preparation technique, such MS techniques as MALDI can be used to release a compound from the matrix, particularly where a labile bond is used originally to tether the compound to the matrix. For instance, a bead selected from a library can be irradiated in a

MALDI step in order to release the diversomer from the matrix, and ionize the diversomer for MS analysis.

*B) Multipin Synthesis*

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The libraries of the subject method can take the multipin library format. Briefly, Geysen and co-workers (Geysen et al. (1984) PNAS 81:3998-4002) introduced a method for generating compound libraries by a parallel synthesis on polyacrylic acid-grated polyethylene pins arrayed in the microtitre plate format. The Geysen technique can be used to synthesize and screen thousands of compounds per week using the multipin method, and the tethered compounds may be reused in many assays. Appropriate linker moieties can

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also been appended to the pins so that the compounds may be cleaved from the supports after synthesis for assessment of purity and further evaluation (c.f., Bray et al. (1990) Tetrahedron Lett 31:5811-5814; Valerio et al. (1991) Anal Biochem 197:168-177; Bray et al. (1991) Tetrahedron Lett 32:6163-6166).

*C) Divide-Couple-Recombine*

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In yet another embodiment, a variegated library of compounds can be provided on a set of beads utilizing the strategy of divide-couple-recombine (see, e.g., Houghten (1985) PNAS 82:5131-5135; and U.S. Patents 4,631,211; 5,440,016; 5,480,971). Briefly, as the name implies, at each synthesis step where degeneracy is introduced into the library, the beads are divided into separate groups equal to the number of different substituents to be

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added at a particular position in the library, the different substituents coupled in separate reactions, and the beads recombined into one pool for the next iteration.

In one embodiment, the divide-couple-recombine strategy can be carried out using an analogous approach to the so-called "tea bag" method first developed by Houghten, where compound synthesis occurs on resin sealed inside porous polypropylene bags

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(Houghten et al. (1986) PNAS 82:5131-5135). Substituents are coupled to the compound-bearing resins by placing the bags in appropriate reaction solutions, while all common steps such as resin washing and deprotection are performed simultaneously in one reaction vessel. At the end of the synthesis, each bag contains a single compound.

*D) Combinatorial Libraries by Light-Directed, Spatially Addressable Parallel Chemical Synthesis*

A scheme of combinatorial synthesis in which the identity of a compound is given by its locations on a synthesis substrate is termed a spatially-addressable synthesis. In one embodiment, the combinatorial process is carried out by controlling the addition of a chemical reagent to specific locations on a solid support (Dower et al. (1991) Annu Rep Med Chem 26:271-280; Fodor, S.P.A. (1991) Science 251:767; Pirrung et al. (1992) U.S. Patent No. 5,143,854; Jacobs et al. (1994) Trends Biotechnol 12:19-26). The spatial resolution of photolithography affords miniaturization. This technique can be carried out through the use of protection/deprotection reactions with photolabile protecting groups.

The key points of this technology are illustrated in Gallop et al. (1994) J Med Chem 37:1233-1251. A synthesis substrate is prepared for coupling through the covalent attachment of photolabile nitroveratryloxycarbonyl (NVOC) protected amino linkers or other photolabile linkers. Light is used to selectively activate a specified region of the synthesis support for coupling. Removal of the photolabile protecting groups by light (deprotection) results in activation of selected areas. After activation, the first of a set of amino acid analogs, each bearing a photolabile protecting group on the amino terminus, is exposed to the entire surface. Coupling only occurs in regions that were addressed by light in the preceding step. The reaction is stopped, the plates washed, and the substrate is again illuminated through a second mask, activating a different region for reaction with a second protected building block. The pattern of masks and the sequence of reactants define the products and their locations. Since this process utilizes photolithography techniques, the number of compounds that can be synthesized is limited only by the number of synthesis sites that can be addressed with appropriate resolution. The position of each compound is precisely known; hence, its interactions with other molecules can be directly assessed.

In a light-directed chemical synthesis, the products depend on the pattern of illumination and on the order of addition of reactants. By varying the lithographic patterns, many different sets of test compounds can be synthesized simultaneously; this characteristic leads to the generation of many different masking strategies.

*E) Encoded Combinatorial Libraries*

In yet another embodiment, the subject method utilizes a compound library provided with an encoded tagging system. A recent improvement in the identification of active compounds from combinatorial libraries employs chemical indexing systems using tags that uniquely encode the reaction steps a given bead has undergone and, by inference, the structure it carries. Conceptually, this approach mimics phage display libraries, where activity derives from expressed peptides, but the structures of the active peptides are deduced from the corresponding genomic DNA sequence. The first encoding of synthetic combinatorial libraries employed DNA as the code. A variety of other forms of encoding have been reported, including encoding with sequenceable bio-oligomers (e.g., oligonucleotides and peptides), and binary encoding with additional non-sequenceable tags.

(1) *Tagging with sequenceable bio-oligomers*: The principle of using oligonucleotides to encode combinatorial synthetic libraries was described in 1992 (Brenner et al. (1992) PNAS 89:5381-5383), and an example of such a library appeared the following year (Needles et al. (1993) PNAS 90:10700-10704). A combinatorial library of nominally $7^7$ (= 823,543) peptides composed of all combinations of Arg, Gln, Phe, Lys, Val, D-Val and Thr (three-letter amino acid code), each of which was encoded by a specific dinucleotide (TA, TC, CT, AT, TT, CA and AC, respectively), was prepared by a series of alternating rounds of peptide and oligonucleotide synthesis on solid support. In this work, the amine linking functionality on the bead was specifically differentiated toward peptide or oligonucleotide synthesis by simultaneously preincubating the beads with reagents that generate protected OH groups for oligonucleotide synthesis and protected $NH_2$ groups for peptide synthesis (here, in a ratio of 1:20). When complete, the tags each consisted of 69-mers, 14 units of which carried the code. The bead-bound library was incubated with a fluorescently labeled antibody, and beads containing bound antibody that fluoresced strongly were harvested by fluorescence-activated cell sorting (FACS). The DNA tags were amplified by PCR and sequenced, and the predicted peptides were synthesized. Following such techniques, compound libraries can be derived for use in the subject method, where the oligonucleotide sequence of the tag identifies the sequential combinatorial reactions that a particular bead underwent, and therefore provides the identity of the compound on the bead.

The use of oligonucleotide tags permits exquisitely sensitive tag analysis. Even so, the method requires careful choice of orthogonal sets of protecting groups required for

alternating co-synthesis of the tag and the library member. Furthermore, the chemical lability of the tag, particularly the phosphate and sugar anomeric linkages, may limit the choice of reagents and conditions that can be employed for the synthesis of non-oligomeric libraries. In preferred embodiments, the libraries employ linkers permitting selective detachment of the test compound library member for assay.

Peptides have also been employed as tagging molecules for combinatorial libraries. Two exemplary approaches are described in the art, both of which employ branched linkers to solid phase upon which coding and ligand strands are alternately elaborated. In the first approach (Kerr JM et al. (1993) J Am Chem Soc 115:2529-2531), orthogonality in synthesis is achieved by employing acid-labile protection for the coding strand and base-labile protection for the compound strand.

In an alternative approach (Nikolaiev et al. (1993) Pept Res 6:161-170), branched linkers are employed so that the coding unit and the test compound can both be attached to the same functional group on the resin. In one embodiment, a cleavable linker can be placed between the branch point and the bead so that cleavage releases a molecule containing both code and the compound (Ptek et al. (1991) Tetrahedron Lett 32:3891-3894). In another embodiment, the cleavable linker can be placed so that the test compound can be selectively separated from the bead, leaving the code behind. This last construct is particularly valuable because it permits screening of the test compound without potential interference of the coding groups. Examples in the art of independent cleavage and sequencing of peptide library members and their corresponding tags has confirmed that the tags can accurately predict the peptide structure.

(2) *Non-sequenceable Tagging -- Binary Encoding*: An alternative form of encoding the test compound library employs a set of non-sequencable electrophoric tagging molecules that are used as a binary code (Ohlmeyer et al. (1993) PNAS 90:10922-10926). Exemplary tags are haloaromatic alkyl ethers that are detectable as their trimethylsilyl ethers at less than femtomolar levels by electron capture gas chromatography (ECGC). Variations in the length of the alkyl chain, as well as the nature and position of the aromatic halide substituents, permit the synthesis of at least 40 such tags, which in principle can encode $2^{40}$ (e.g., upwards of $10^{12}$) different molecules. In the original report (Ohlmeyer et al., supra) the tags were bound to about 1% of the available amine groups of a peptide library via a photocleavable o-nitrobenzyl linker. This approach is convenient when preparing

combinatorial libraries of peptide-like or other amine-containing molecules. A more versatile system has, however, been developed that permits encoding of essentially any combinatorial library. Here, the compound would be attached to the solid support via the photocleavable linker and the tag is attached through a catechol ether linker via carbene

5      insertion into the bead matrix (Nestler et al. (1994) J Org Chem 59:4723-4724). This orthogonal attachment strategy permits the selective detachment of library members for assay in solution and subsequent decoding by ECGC after oxidative detachment of the tag sets.

Although several amide-linked libraries in the art employ binary encoding with the

10     electrophoric tags attached to amine groups, attaching these tags directly to the bead matrix provides far greater versatility in the structures that can be prepared in encoded combinatorial libraries. Attached in this way, the tags and their linker are nearly as unreactive as the bead matrix itself. Two binary-encoded combinatorial libraries have been reported where the electrophoric tags are attached directly to the solid phase (Ohlmeyer et

15     al. (1995) PNAS 92:6027-6031) and provide guidance for generating the subject compound library. Both libraries were constructed using an orthogonal attachment strategy in which the library member was linked to the solid support by a photolabile linker and the tags were attached through a linker cleavable only by vigorous oxidation. Because the library members can be repetitively partially photoeluted from the solid support, library members

20     can be utilized in multiple assays. Successive photoelution also permits a very high throughput iterative screening strategy: first, multiple beads are placed in 96-well microtiter plates; second, compounds are partially detached and transferred to assay plates; third, a metal binding assay identifies the active wells; fourth, the corresponding beads are rearrayed singly into new microtiter plates; fifth, single active compounds are identified;

25     and sixth, the structures are decoded.

### *Exemplification*

The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration

30     of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

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### Example 1

*1. Synthesis of N-α-(tert-Butoxycarbonyl)-N-ω-bis(2-pyridylmethyl)-L-lysine (**L1c-Boc**)*

2-Chloromethylpyridine hydrochloride (1.4g, 8.53 mmol) and N-α-(tert-
5  Butoxycarbonyl)-L-lysine (1g, 4.06 mmol) were dissolved in water and stirred at room
temperature for five days, with addition of 5 mol dm$^{-3}$ aqueous NaOH solution at intervals
to maintain the pH at 8-10. The resulting dark red solution was extracted with ethyl acetate,
and then the aqueous phase was acidified to pH 3-4 by 1 mol dm$^{-3}$ HCl and extracted with
Chloroform and concentrated. This residue purified by column chromatography using 10%
10 chloroform in methanol to give N-α-(tert-Butoxycarbonyl)-N-ω-bis(2-pyridylmethyl)-L-
lysine (950 mg, 55%). $^{1}$H NMR (CDCl$_3$), 300 MHz): 1.41 (s, 9H), 1.26-1.62 (m, 6 H), 2.58
(t, 2H), 3.84 (s, 4H), 4.24 (t, H), 7.15 (m, 2H), 7.48 (d, 2H), 7.65 (m, 2H), 8.53 (d, 2H). $^{13}$C
NMR (CD$_3$OD, 300 MHz): 24.31 (C, CH$_2$), 26.66 (C, CH$_2$), 28.93 (3C, t-Bu), 33.15 (C,
CH$_2$), 55.50 (C, NCH$_2$), 60.12 (2C, PyCH$_2$), 80.06 (C, NCH) 124.34 (2C, Py), 125.11
15 (2CH, Py), 138.93 (2CH, Py), 149.72 (2CH, Py), 157.71 (2C, Py), 177.49 (C, CO$_2$H).

### Example 2

*1. Synthesis of N-α-(2-pyridylmethyl)-N-ω-(tert-Butoxycarbonyl)-L-lysine (**L2d-Boc**)*

2-Chloromethylpyridine hydrochloride (730mg, 4.46 mmol) and N-α-(tert-
20 Butoxycarbonyl)-L-lysine (1g, 4.06 mmol) were dissolved in water and stirred at room
temperature for two days, with addition of 5 mol dm$^{-3}$ aqueous NaOH solution at intervals
to maintain the pH at 8-10. The resulting dark red solution was extracted with ethyl acetate,
and then the aqueous phase was acidified to pH 6 by 1 mol dm$^{-3}$ HCl and followed by
treating with chloroform the required product precipitate out, which was filtered and dried
25 under vacuum (670mg, 49%).

### Example 3

*1. Labeling the DPMA analogs with Tc-99m using labeling methods
based on the Tc(V)-oxo and Tc(I)(CO)$_3$L$_3$ cores*

*(a) Tc(V)-oxo core:* Preparation of the Tc-99m-labeled DPMA derivatives was achieved by adding 10 mCi of $TcO_4^-$ to a 0.9% saline solution of the DPMA derivative (200 mg/3 mL). The mixture was heated at 80 °C for 30 min. Depending on the biological ligand, the solution was used as needed or the mixture was extracted with ethyl acetate (3, 1

5      mL portions), dried over sodium sulfate, and dried under $N_2$. The residue was then re-dissolved in ethanol (400 uL) and purity checked via HPLC by a Vydac C18 (5 mm, 25 cm) column using methanol to elute the reaction products.

*(b) Tc(I)(CO)$_3^+$ core:* The Tc(I) carbonyl chemistry allows for the possibility of an alternative route to form stable $^{99m}$Tc-DPMA complexes. To explore this labeling method

10     we began by placing $Na_2CO_3$ (0.004 g, 0.038 mmol), $NaBH_4$ (0.005 g, 0.13 mmol), and 2 mg of the DPMA derivative in a vial. Next, the vial was sealed and flushed with CO for 10 min. To the vial was added 1 mL of Na $^{99m}$TcO$_4^-$ in saline. Finally the solution was heated to 100° C for 30 minutes. After cooling, the reaction was then checked for purity via HPLC by a Vydac C18 (5 mm, 25 cm) column using methanol to elute the reaction products.

15         Alternatively, a 'two pot' synthesis could be performed, where the DPMA derivative was added after the formation of $[^{99m}Tc(OH_2)_3(CO)_3]^+$. After cooling, 0.3 mL of 1 M PBS solution was added (pH 7.4), resulting in the stable formation of $[^{99m}Tc(OH_2)_3(CO)_3]^+$. This Tc(I) tricarbonyl species was then heated at 75° C for 30 minutes with the DPMA derivative to form the $^{99m}$Tc-DPMA complex. The reaction was then checked for purity via HPLC by

20     a Vydac C18 (5 mm, 25 cm) column using methanol to elute the reaction products. The versatility of the reaction allows for the reaction of a variety of sensitive biological DPMA derivatized ligands to be kept under idealized conditions.

## *Example 4*

25     *1. Synthesis of [{N,N-di(pyridyl-2-methyl)}N-butyl-phthalimide] and $^{99m}$Tc labeling*

The dipyridinemethylamine (0.5 g, 2.51 mmol) and N-(4-bromobutyl)-phthalimide (0.85 g, 3.02 mmol) were mixed in a 100 mL pressure tube in 2 mL of DMF. Potassium carbonate (0.05 g) was added to the solution. The mixture was heated at 120 C for 1 hr. The reaction mixture was vacuumed down to residue. The residue was purified through a

30     pad of silica gel using methanol-methylene chloride to provide the product in 41 % yield. $^1$H NMR(CDCl$_3$): 1.57 (m), 2.54 (m), 2.85 (s), 2.93 (s), 3.58 (m), 3.76 (s), 7.09 (m), 7.52
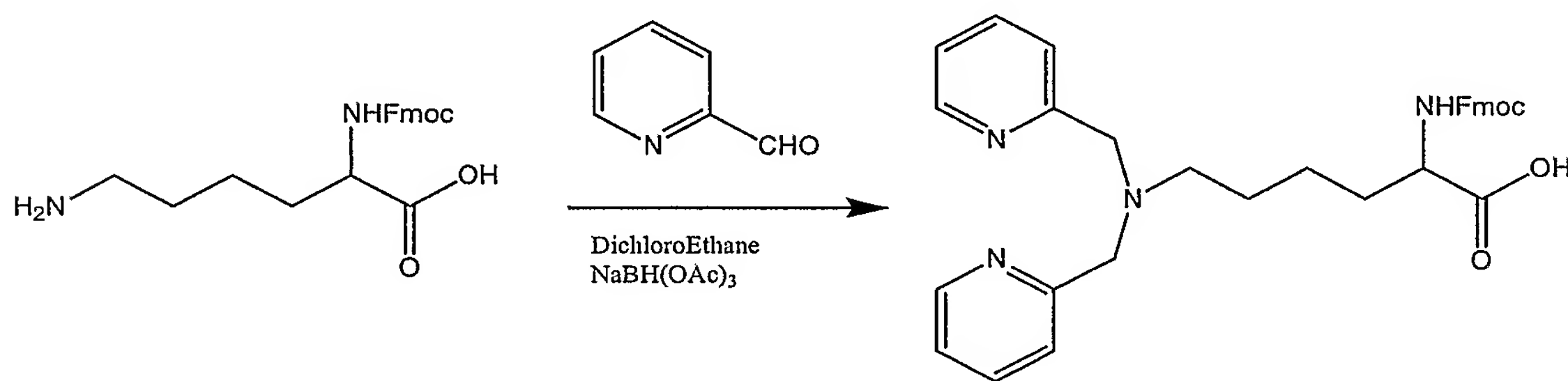
(d), 7.61 (m), 7.68 (m), 7.80 (m), 7.99 (d), 8.44 (d).

$[^{99m}Tc(CO)_3(H_2O)_3]^+$ was heated with [{$N,N$-di(pyridyl-2-methyl)}$N$-butyl-phthalimide in 0.5 mL (1 mg/mL) of methanol at 100° C for 60 minutes. Purity, analyzed via C18 HPLC, showed >99% RCY. The product eluted with methanol at 20.8 minutes.

5    The HPLC analysis was performed using a Supelco C18 column, 25cm x 4.6mm column (5μm pore size), equipped with 2 cm guard using solvent A = 0.05 M triethylammonium phosphate buffer pH 2.5  and solvent B = methanol.  The method employed was a gradient 5-95% B, 1 mL/ minute for 30 minutes.  The gradient ramped from 5-95 from 3-20 minutes. In challenge experiments the HPLC purified product demonstrated no degradation in either

10   10mM Cysteine or Histidine in PBS pH 7.2 at 37° C for 20 hrs.

## *Example 5*

*1. [ε-{N,N-di(pyridyl-2-methyl)}α-(fmoc)lysine] (Fmoc-DpK; 1)*



15          The fmoc-lysine, 2-pyridinecarboxaldehyde and sodium triacetoxyborohydride were mixed in 1,2-dichloroethane. The suspension was stirred at ambient temperature under an argon atmosphere for 1 hr. The reaction mixture was portioned between chloroform and water. The residue was purified through a pad of silica gel using methanol-chloroform  to provide the product in 85 % yield.  Fmoc-deprotection employed stirring 4-

20   dimethylaminopyridine in DMF/methanol at 25 °C for 12 hrs. Structural confirmation was performed by $^1$H and $^{13}$C NMR. $^1$H NMR (δ (ppm), CDCl$_3$): 10.85 (bs, 1H, CO$_2$H), 8.50 (d, $J$ = 5.10 Hz, 2H, PyH), 7.70 (d, $J$ = 7.24 Hz, 2H, FlH), 7.55 (m, 4H, PyH, FlH), 7.46 (d, $J$ =7.24, 2H, FlH), 7.32 (t, $J$ =7.72, 2H, Py), 7.22(t, $J$ =7.52, 2H, Py), 7.09 (t, $J$ =6.20, 2H, FlH), 6.0 (d, $J$ =9.31, 1H, NH), 4.29 (m, 3H, OCH$_2$ , NCHCO$_2$), 4.17 (t, $J$ =6.20,1H, CH),

25   3.86 (s, 4H, PyCH$_2$), 2.57 (t, 2H, NCH$_2$), 1.90-1.20 (m, 6H, CH$_2$). $^{13}$C NMR ((δ (ppm), CDCl$_3$): 175.96 (C, CO$_2$H), 157.74 (2C, Py), 156.15 (C, CONH), 148.29 (2CH, Py), 144.12

(2C, Fl), 141.27(2C, Fl), 137.38 (2CH, Py), 127.68 (2CH, Py), 127.08 (2CH, Py), 125.26(2CH, Fl), 123.92(2CH, Fl), 122.64(2CH, Fl), 119.96(2CH, Fl), 66.81(1C, OCH$_2$), 59.03 (2C, PyCH$_2$), 54.48 (C, NCHCO$_2$), 53.87 (C, NCH$_2$), 47.24 (C, Fl), 32.54 (C, CH$_2$), 26.04(C, CH$_2$), 22.86(C, CH$_2$).

2. *[Re(CO)$_3${$\eta^3$-$\varepsilon$-[(N,N-di(pyridyl-2-methyl)] $\alpha$(fmoc) lysine}] [Br]*

To a stirred solution of [NEt$_4$]$_2$[Re(CO)$_3$Br$_3$] (1.12 g, 1.45 mmol) in methanol (20 mL) was added [$\varepsilon$-{*N,N*-di(pyridyl-2-methyl)}$\alpha$-(fmoc)lysine] (0.8 g, 1.45 mmol) in 2 mL methanol, whereupon the solution was refluxed for 5 hr and concentrated. The residue was dissolved in chloroform, washed with water, dried (NaSO$_4$) and evaporated to dryness to give a colorless product (1.04 g, 80%). $^1$H NMR ($\delta$(ppm), MeOH-*d$_4$*): 8.88(d, *J*=5.29, 2H), 8.02-7.37 (m, 14H),5.05 (d, *J* = 17.64 Hz, 2H, PyCH$_2$), 4.82 (d, *J* = 17.64 Hz, 2H, PyCH$_2$), 4.44-4.35 (m, 4H), 3.88 (m, 2H), 2.20-1.50 (m, 6H, CH$_2$). $^{13}$C NMR ($\delta$(ppm), MeOH-*d$_4$*): 197.47, 196.44 (*fac*-Re-CO$_3$), 175.42 (C, CO$_2$H), 161.82 (2C, Py), 158.30(C, CONH),152.87 (2CH, Py), 145.13 (2C, FlH),142.29 (2C, FlH), 141.48 (2CH, Py), 129.07 (2CH, Py), 128.46 (2CH, Py), 126.94 (2CH, FlH), 126.58(2CH, FlH), 124.83(2CH, FlH), 121.23(2CH, FlH), 71.66 (NCH$_2$), 68.72 (2C, PyCH$_2$), 67.70 (C, OCH$_2$), 55.27(NCHCO$_2$), 32.15(C, CH$_2$), 25.71 (2C,CH$_2$), 24.39(C, CH$_2$).

*3. Tc-99m labeling*

[$^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ was heated with [$\varepsilon$-{*N,N*-di(pyridyl-2-methyl)}$\alpha$-(fmoc)lysine] (DpK) in 0.5 mL (1 mg/mL) of methanol at 100° C for 30 minutes. Purity, analyzed via C18 HPLC, showed >99% RCY. In challenge experiments the HPLC purified product demonstrated no degradation in either 100 mM Cysteine or Histidine in PBS pH 7.2 at 37° C for 18 hrs. Labeling yields of > 50% RCY, were achievable at levels as low as 2 μg / mL.

**Table 1.** Labeling results of Tc99m-DpK Complexes.

| Ligand Amounts ( μg ) | % Labeled Fmoc-DpK | % Labeled DpK |
|:---:|:---:|:---:|
| 500 | 100 | 100 |
| 100 | 100 | 47 |
| 10 | 93.9 | 32 |
| 1 | 52 | 16 |
| 0.1 | 7 | 5 |

### *Example 6*

1. *[CuCl{$\eta^3$-$\varepsilon$-[(N,N-di(pyridyl-2-methyl)] $\alpha$(fmoc) lysine}]*

To a solution of CuCl$_2$ in 10 mL of methanol was added an excess of Fmoc protected dipyridine lysine (Fmoc-DpK). The solution was heated at 150 C for 3 hours in a 100ml sealed pressure tube. Upon completion the solution was cooled and vaccuumed down to residue. The residue was dissolved in methylene chloride and layered with ether. After 12 hours a dark green-blue oil formed. The oil was sent out for ES/MS resulting in an observed masses of 648-650, which corresponds to the [CuCl(DpK)] complex. The oily product was cleaned up using a Waters C18 sep pak using 10% ethanol/ H$_2$O for the load. The purified product weighed 60 mg for 81% yield. $^1$H NMR (CDCl$_3$, 300 mhz, ppm) was performed: 1.23(m), 3.71(d), 3.83 (m), 4.19 (m), 4.35 (s), 7.13 (m), 7.26 (m), 7.35 (m), 7.46 (m), 7.51 (m), 7.61 (m), 7.72(m), 8.51(s). HPLC analysis was performed on a Vydac C18 column, 25cm x 4.6mm column (5μm pore size), equipped with 2 cm guard using solvent A = H2O + 0.1% TFA B= CH3CN + 0.1% TFA. The method employed was a gradient 15-80% B, 1 mL/ minute for 30 minutes. The gradient ramped from 15-80 from 3-22 minutes. The product eluted as two peaks (racemic mix of DpK ligand) at 19.3 and 19.6 minutes.

2. *[$^{64}$CuCl{$\eta^3$-$\varepsilon$-[(N,N-di(pyridyl-2-methyl)] $\alpha$(fmoc) lysine}]*

$^{64}$CuCl$_2$ was heated with Fmoc protected dipyridine lysine (Fmoc-DpK) in 0.5 mL (100 μg/mL) of methanol at 70° C for 20 minutes. Purity, analyzed via C18 HPLC, showed >85% RCY. The product eluted at 19.8 minutes.

## *Example 7*

### *1. Peptide Synthesis and Characterization (Prophetic)*

Peptides are prepared on an Advanced ChemTech 348 Ω Peptide Synthesizer using

5   HBTU as the coupling agent (*see* Figure 2) (Chan, W.C., White, P.D. FMOC Solid-Phase Peptide Synthesis, A Practical Approach. Oxford University Press: New York, 2000; Fields, G. B., Noble, R. L. (1990) Solid-phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acids. *Int. J. Peptide Protein. Res. 35*, 161-214; and Fields, C.G., Lloyd, D.H., Macdonald, R.L., Otteson, K.M., Noble, R.L. (1991) HBTU

10  Activation for automated Fmoc solid-phase peptide synthesis. *Pept. Res. 4*, 95-101). The Fmoc protected chelate or its organometallic complex, as the bromide salt, are dissolved in DMF and coupled to the growing peptide chain using about a 4-fold excess of ligand. The duration of the coupling steps to afford complete conversion is determined by exposing samples of resin taken from the reaction mixtures to a solution containing ninhydrin. The

15  time to complete conversion of the amine to the amide in both cases is identical to the conditions used for natural amino acid derivatives. As a result, modification of standard peptide coupling protocols is not necessary. Peptides are cleaved from the resin using a TFA solution containing ethanedithiol (EDT, 2 %), water (2 %), and triisopropylsilane (TIS, 2 %). Because of the presence of methionine, exclusion of oxygen and the use of freshly

20  distilled EDT is necessary to avoid oxidation of the thioether to the sulfoxide. Precipitation of the peptide TFA salts is brought about by trituration with cold ether. The resulting solids are collected by centrifugation and washed with cold ether. Following dissolution in distilled water and lyophilization, compounds are collected as solids.

25                                                    *Example 8*

### *1. Animal Studies (Prophetic)*

(a) *Biodistribution of Tc-99m-Heteroaryl Compounds of the Invention:* The biodistribution of Tc-99m bound compounds of the instant invention are investigated in male rats (Sprague Dawley, n = 5 / timepoint, ~180 gms). The compounds are injected via

30  the tail vein in saline (*e.g.* 10 μCi / 100 μl). Animals are sacrificed at 5, 30, 60 and 120 minutes p.i. and distribution is measured, for example, in the blood, heart, lung, liver,

kidney, and GI track.

(b) *Heart Imaging Agents:* Vertebrate animals (*e.g.*, rats) are used to investigate the biodistribution and pharmacokinetics of the new technetium complexes and uptake in the heart is determined. Rats (Sprague Dawley, male, at 80-100 grams each) are used for whole
5 body biodistribution studies. The compounds are evaluated at two time points, *i.e.*, 5 and 60 minutes, with four animals per time point. The use of this number of animals provides accurate statistics in the clearance rate measurements, and accounts for intraspecies variation.
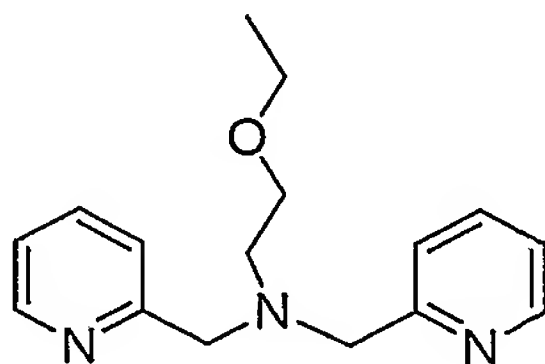
10                                                      *Example 8*

*1. Synthesis*

        All HPLC experiments were performed on a Varian Prostar HPLC equipped with Autosampler (Model 410), UV-visible detector (Model 345), NaI radiometic detector, and Prostar Pumps (model 210). The preparation of the 0.05 M Triethylammonium phosphate
15 pH 2.25 HPLC solvent was performed by adding 7 mL of triethylamine to 500 mL of $H_2O$. This was followed by the addition of 4 mL of phosphoric acid to reach the desired 2.25 pH. The solution was diluted to 1000 mL with $H_2O$ and filtered through a 0.22 μm cellulose filter into a 1 liter HPLC bottle. The solution was sonicated for 10 minutes to degas.

        Technetium-99m was used as a $Na^{99m}TcO_4$ solution in saline, as a commercial
20 $^{99}Mo/^{99m}Tc$ generator eluant (Cardinal Health). Technetium-99m ($^{99m}Tc$) is a γ emitter (141 keV) with a half-life of 6h. The $^{99m}Tc$-containing solutions were always kept behind sufficient lead shielding. The use of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared from commercially available Isolink™ kits (Mallinckrodt). The Tc-99m-complexes were prepared and injected as a 10% ethanol / saline solutions.
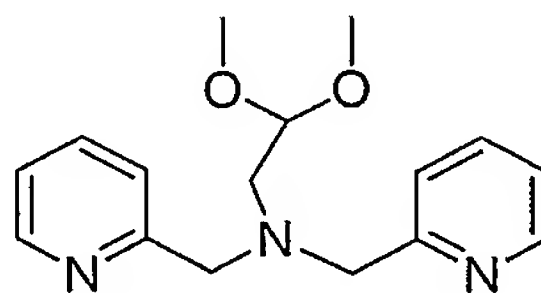
25 *1.1. [N-ethyl-ethoxy-dipyridine-2-methylamine]* **(8)**



        Placed 2-di-(picoline)amine (0.50 g, 2.51 mmol) and 1-bromoethyl-ethoxy(0.420 g,

2.76 mmol) in a 100 mL pressure tube with a stir bar. The solids were dissolved in 2 mL of dried DMF. Potassium carbonate (0.05 g, 0.362 mmol) and NEt₃ (1 mL) were added to the solution. The solution was heated at 125 °C for 4 hrs. and then vacuumed down to residue. The residue was passed through a silca gel column using 2% methanol / methylene chloride as the solvents. The product was eluted as a yellow oil (0.568 g, 83.3 %). $^1$H NMR (CDCl₃), 300 MHz): 1.12 (t, 3H), 2.79 (t, H), 2.84 (s, 2H), 2.91 (s, 2H), 3.39 (q, H), 3.52 (t, H), 3.87 (s, 2H), 5.24 (s, H), 7.11 (t, 2H), 7.54 (m, 2H), 7.60 (m, H), 7.97 (s, H), 8.47 (d, 2H). GCMS = M.W. 273. Calc. M.W. = 272.

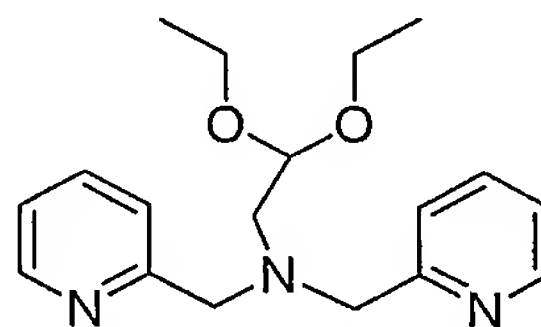*1.2. [N-ethyl-dimethoxy-dipyridine-2-methylamine]* (**9**)



The dipyridine-2-methylamine (0.50 g, 2.51 mmol) was placed in a 15 mL pressure tube equipped with a stirrer. The solution was dissolved in 3 mL of DMF, 2 mL of triethylamine, followed by addition of potassium carbonate (0.10 g, 0.72 mmol), and the 2-bromo-1, 1-dimethoxy-ethane (0.47 g, 2.76 mmol). The solution was heated at 110 °C for 1 hrs. The solution was then vacuumed down to residue. The residue was passed through a HPLC silca gel column using 0-10% methanol / methylene chloride as the solvents, yielding 0.25 g, 34.7% yield. $^1$H NMR ((CDCl₃), 300 MHz): 2.77 (d, 2H), 3.28 (s, 6H), 3.92 (s, 4H), 4.53 (t, H), 7.12 (t, 2H), 7.55 (d, 2H), 7.65 (m, 2H), 8.51 (d, 2H), GC/MS = 288 (M+1).

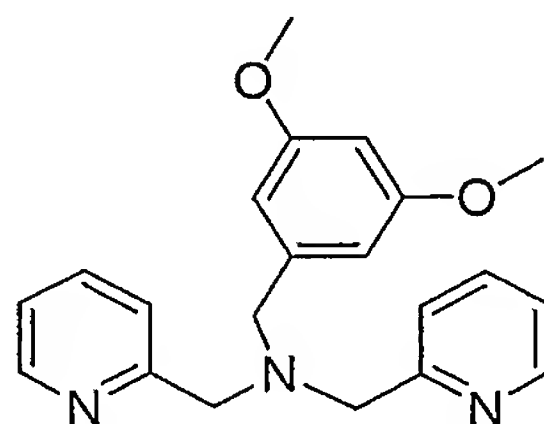*1.3. [N-ethyl-diethoxy-dipyridine-2-methylamine]* (**10**)



The dipyridine-2-methylamine (0.50 g, 2.51 mmol) was placed in a 15 mL pressure tube equipped with a stirrer. The solution was dissolved in 3 mL of DMF, 2 mL of triethylamine, followed by addition of potassium carbonate (0.10 g, 0.72 mmol), and the 2-bromo-1, 1-diethoxy-ethane (0.54 g, 2.76 mmol). The solution was heated at 130 °C for 1 hrs. The solution was then vacuumed down to residue. The residue was passed through a HPLC silca gel column using 0-10% methanol / methylene chloride as the solvents, yielding

0.51 g, 64.6% yield. $^1$H NMR ((CDCl$_3$), 300 MHz): 1.14 (t, 6H), 2.77 (d, 2H), 3.44 (m, 2H), 3.59 (m, 2H), 3.92 (s, 4H), 4.63 (t, H), 7.11 (dd, 2H), 7.56 (d, 2H), 7.64 (m, 2H), 8.48 (d, 2H), GC/MS = 316.
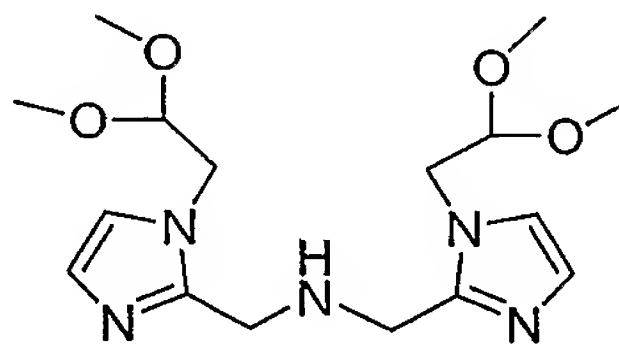
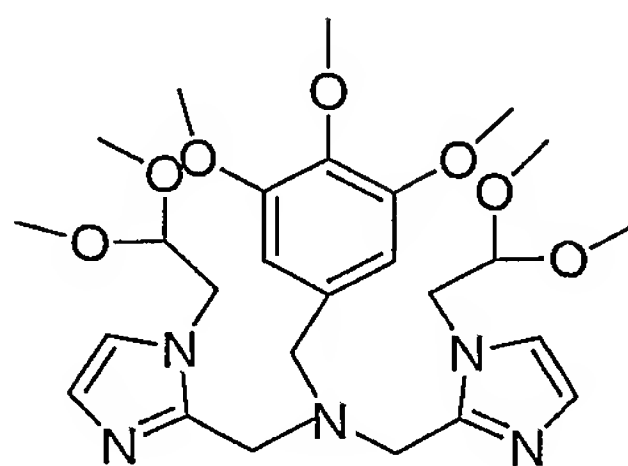*1.4. [N-3, 5-dimethoxybenzyl -dipyridine-2-methylamine]* **(11)**



Placed 2-di-(picoline)amine (0.50 g, 2.51 mmol) and 3, 5-dimethoxybenzyl bromide (0.698 g, 3.02 mmol) in a 100 mL pressure tube with a stir bar. The solids were dissolved in 2 mL of dried DMF. Potassium carbonate (0.05 g, 0.362 mmol) and NEt$_3$ (1mL) were added to the solution. The solution was heated at 125 °C for 1.5 hrs. and then vacuumed down to residue. The residue was passed through a silca gel column using 2% methanol / methylene chloride as the solvents. The product was eluted as a yellow oil (0.50 g, 57.1 %). $^1$H NMR (CDCl$_3$), 300 MHz): 2.83 (s, 2H), 2.89 (s, 2H), 3.61 (s, 2H), 3.74 (s, 3H), 3.78 (s, 3H), 6.31 (t, H), 6.58 (d, 2H), 7.09 (t, 2H), 7.59 (m, 4H), 8.47 (d, 2H). GCMS = M.W. 351. Calc. M.W. = 349.

*1.5. [N-{ethyl-2-dimethoxy}-2-imidazolecarboxaldehyde]* **(12)**



The 2-imidazolecarboxaldehyde (2.0 g, 0.021 mol) was placed in a 15 mL pressure tube equipped with a stirrer under argon. The solution was dissolved in 2 mL of DMF, followed by addition of potassium carbonate (0.50 g, 3.6 mmol), and bromoacetaldehyde dimethyl acetal (03.87 g, 0.023 mmol). The solution was heated at 120 °C for 20 hrs. The solution was then vacuumed down to residue. The residue was passed through a HPLC silca gel column using 0-10% methanol / methylene chloride as the solvents, yielding 1.15 g, 30.1% yield. $^1$H NMR (CDCl$_3$), 300 MHz): 3.37 (s, 6H), 4.47 (m, 2H), 7.20 (s, H), 7.25 (s, H), 9.78 (s, H).

*1.6.  [N-{ethyl-2-dimethoxy}-2-methyl-imidazole-3,4,5-trimethoxy-benzylamine]* (**13**)



Placed 3,4,5-trimethoxy-benzylamine (0.054 g, 0.027 mol) in a 100 mL round-
bottom flask equipped with a stirrer under nitrogen. The liquid was dissolved in 8 mL of
5    dichloroethane, followed by addition of [N-{ethyl-2-dimethoxy}-2-imidazolecarbox-
aldehyde]  (0.10 g, 0.054 mmol) and sodium triacetoxyborohydride (0.127 g, 0.059 mmol).
The solution was stirred at room temperature for 18 hours. The solution was then vacuumed
down to residue. The residue was passed through a HPLC silca gel column using 0-5%
methanol / methylene chloride as the solvents, yielding 0.124 g, 85.5% yield. $^1$H NMR
10   ((CDCl$_3$), 300 MHz): 3.21 (s, 12H), 3.54 (s, 2H), 3.72 (s, 4H), 3.80 (s, 3H), 3.82 (s, 6H),
3.87 (d, 4H), 4.20 (t, 2H), 6.43 (s, 2H),, 6.92 (d, 2H). GCMS = 535 (M+1).

*2. Technetium-99m labeling* (**Tc-8** to **Tc-13**)

The technetium labeling was accomplished using the Tc(I)-tricarbonyl methods. The
Tc(I)(CO)$_3$$^+$ core was readily formed using the Isolink™ kit (Mallinkrodt).  The
15   [$^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ starting material was formed by adding 1 mL of TcO$_4$$^-$ in saline to an
Isolink™ kit.  The solution was heated at 100°C for 30 minutes, followed by the addition of
120 μl of 1N HCl to neutralize the solution.  The [$^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ (200 μl) was added
to the appropriate derivative in 0.2 mL (1mg / mL) of methanol and heated at 80° C for 1
hour.

20   Analysis of the reaction products using C18 HPLC, showed >60% RCP for all
complexes. The HPLC analysis was performed using a Vydac C18 column, 25cm x 4.6mm
column (5μm pore size), equipped with a 2 cm guard column. Solvent A was 0.05 M
triethylammonium phosphate buffer pH 2.5 and solvent B was methanol.  The method
employed a gradient run over 30 minutes at a flow rate of 1ml / minute.  The gradient
25   ramped from 5-100% B from 3-20 minutes.
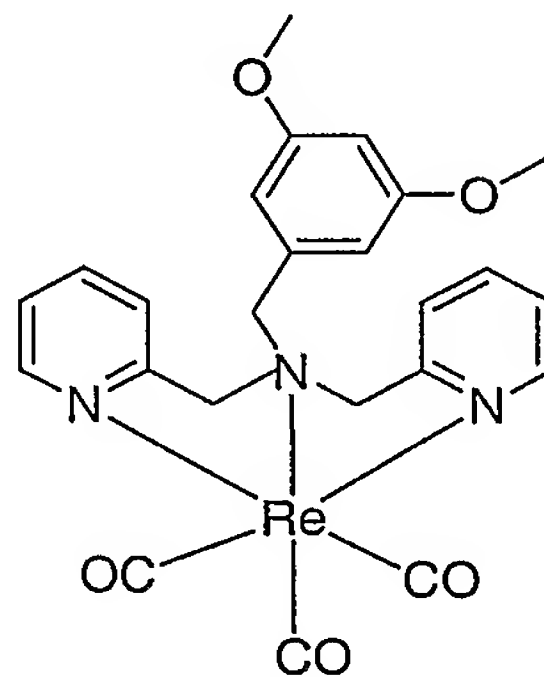
*3. Animal Studies (Prophetic)*

Vertebrate animals are used to investigate the biodistribution and pharmacokinetics

of the radionuclide-bound quinoline and isoquinoline derivatives and determine their uptake in the heart. Rats (Sprague Dawley, male, at 80-100 grams each) are used for the whole body biodistribution studies. The Tc-complexes, as well as Cardiolite™, are evaluated at three time points; 5, 30, and 120 minutes, with five animals per time point. In order to

5    provide accurate statistics in the clearance rate measurements and to account for intra-species variation it is necessary to use this number of animals. The product is diluted to ~10μCi / 100 μl using freshly prepared 10% ethanol / saline (0.9%) solution. The rats are injected via a lateral tail vein with a volume of 0.1 mL. The rats are then sacrificed by decapitation, with immediate blood collection at the desired time points. Whole body

10   biodistributions are performed on the animals immediately following decapitation, organ and tissue samples are taken and washed of excess blood, blotted dry and weighed. Radioactivity is assayed using automated NaI well counter. All tissue samples are counted together along with an aliquot of the injected dose so that % injected dose and % injected dose per gram of tissue could be calculated.

15       *See* Boschi, A. *et al.* Synthesis and Biological Evaluation of Monocationic Asymmetric 99mTc-Nitride Heterocomplexes Showing High Heart Uptake and Improved Imaging Properties. J. Nucl. Med. (2003) 44: 806-814.

### *Example 9*

20   *1.1 [Re(CO)₃(N-3,5-dimethoxybenzyl-dipyridine-2-methylamine)]* (**Re-11**)
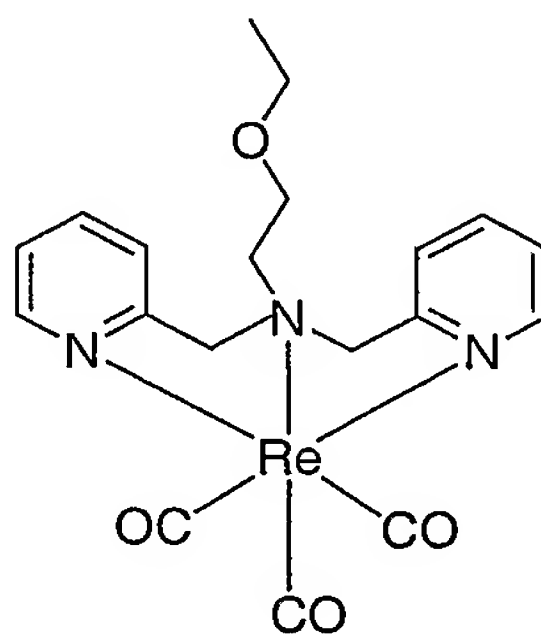


The [NEt₄]₂[Re(CO)₃(H₂O)₃] (0.015 g, 0.019 mmol) and 2-di(picoline)amine-*N*-3, 5-dimethoxybenzyl (KM08-121) (0.0068 g, 0.019 mmol) were placed in a 100 mL pressure tube with a stirr bar. The solids were dissolved in 5 mL of methanol. The solution was

25   heated at 130 °C for 3 hrs. The solution was vacuumed down to residue. The residue was

passed through a silca gel column using 10% methanol / methylene chloride as the solvents. The product eluted as the rhenium complex (11.3 mg, 91.5 %). $^1$H NMR (CDCl$_3$), 300 MHz): 1.17 (s, H), 1.56 (s, 3H), 3.47 (d, H), 3.87 (s, 3H), 4.64 (m, 2H), 5.73 (d, 2H), 6.59 (t, H), 6.75 (d, H), 7.16 (t, 2H), 7.31 (m, H), 7.80 (t, 2H), 7.95 (d, 2H), 8.62 (d, 2H). LC/MS = M.W. 620. Calc. M.W. = 619.
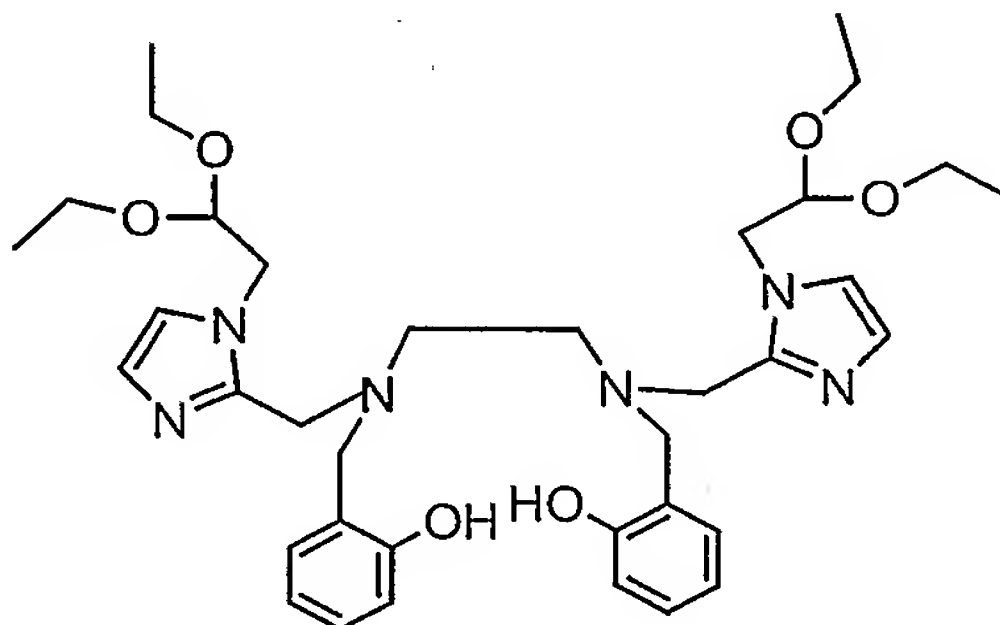
*1.2 [Re(CO)$_3$(N-ethyl-ethoxy-dipyridine-2-methylamine)]* **(Re-8)**



The [NEt$_4$]$_2$[Re(CO)$_3$(H$_2$O)$_3$] (0.04 g, 0.052 mmol) and 2-di(picoline)amine-*N*-ethyl ethoxy (KM08-131) (0.014 g, 0.052 mmol) were placed in a 100 mL pressure tube with a stirr bar. The solids were dissolved in 5 mL of methanol. The solution was heated at 130 °C for 2 hrs. The solution was vacuumed down to residue. The residue was passed through a silca gel column using 10% methanol / methylene chloride as the solvents. The product eluted as the rhenium complex (8 mg, 28.6 %). $^1$H NMR (CDCl$_3$), 300 MHz): 1.25 (t, 3H), 3.72 (d, 2H), 3.97 (t, 2H), 4.05 (t, 2H), 4.55 (d, 2H), 6.10 (d, 2H), 7.18 (t, 2H), 7.80 (t, 2H), 7.95 (d, 2H), 8.62 (d, 2H). LC/MS = M.W. 542.3 Calc. M.W. = 542.2.

*Example 10*

*1. Synthesis of [N, N'-{N-ethyl-2-diethoxy}-2-methyl-imidazole}-N,N'-bis(2-hydroxybenzyl)-ethylenediamine]*
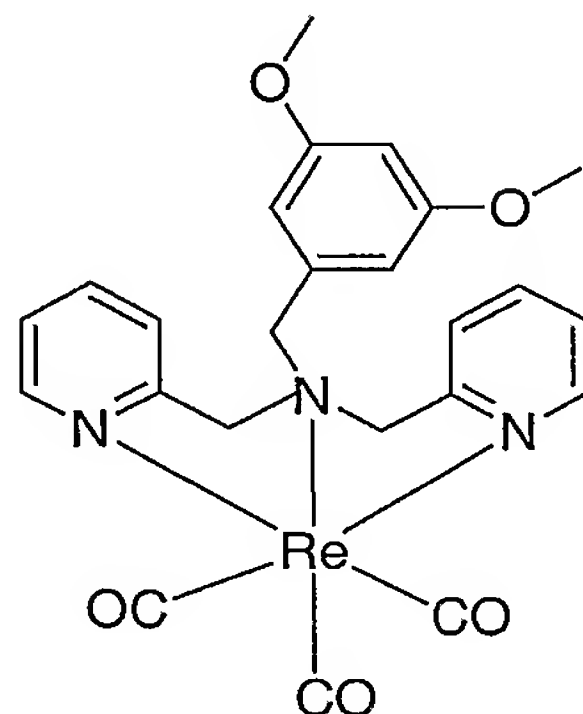
Placed N, N'-bis(2-hydroxybenzyl)ethylenediamine (0.1 g, 0..367 mmol) in a 100

ml round-bottom flask equipped with a stirrer under nitrogen. The solid was dissolved in 8

ml of dichloroethane, followed by addition of [N-{ethyl-2-diethoxy}-2-

imidazolecarboxaldehyde] (0.165 g, 0.775 mmol) and sodium triacetoxyborohydride (0.178

g, 0.845 mmol). The solution was stirred at room temperature for 18 hours. The solution

was then vacuumed down to residue. The residue was passed through a silca gel column

using 0-20% methanol / methylene chloride as the solvents, yielding 0.049 g, 20.1% yield.

$^1$H NMR ((CDCl$_3$), 300 MHz): 1.10 (t, 12H), 2.10 (s, 8H), 2.77 (s, 2H), 3.34 (m, 4H), 3.58

(m, 4H), 3.77 (s, 2H), 3.84 (d, 2H), 4.45 (t, 2H), 6.72 (t, 2H), 6.85 (m, 8H), 6.96 (s, 2H),

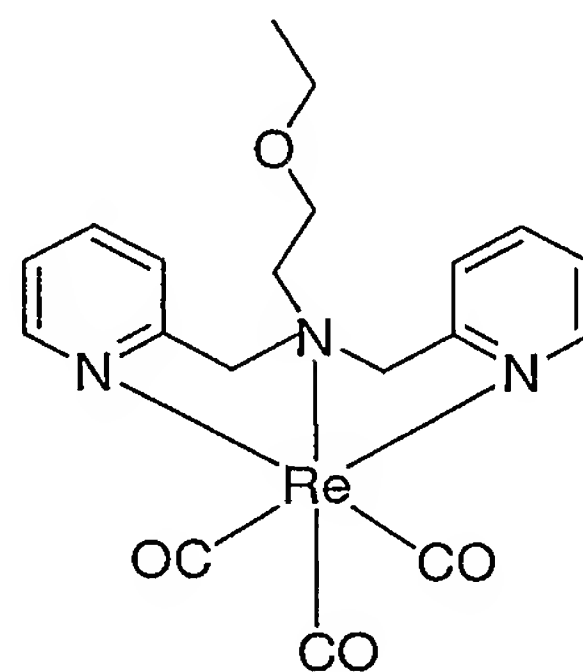7.13 (t, 2H). ES/MS = 666-668: expected 668.


## *Example 11*

*1. Ethyl [(2-Pyridylmethyl)-2-(1-methylimidazolylmethyl)]aminoacetate ($L^{10}Et$).*

To a solution of 1-methylimidazole-2-aldehyde (5 g, 45.1 mmol) in 80 mL of

methanol was added slowly a solution of 2-picolylamine (4.88 g, 45.1 mmol) in 20 mL of

methanol, andthe solution was stirred for 2 h. At this time, the reactants were completely

consumed. To this reaction mixture was added NaBH$_4$ (1.7 g, 45.1 mmol) in portions, and

the solution was stirred for another 3 h, whereupon the solution was evaporated to dryness

and the residue was extracted with chloroform and concentrated. This residue was dissolved

in anhydrous dimethylformamide (40 mL). Potassium carbonate (7.53 g, 45.1 mmol) and

ethyl bromoacetate (6.23 g, 45.1 mmol) were added to the solution under an argon

atmosphere. The resulting suspension was protected from light and allowed to stir at 30 °C,

under argon, for 32 h. The reaction mixture was filtered, and the filtrate was evaporated to

dryness. The resulting red oil was purified by silica gel column chromatography using a

MeOH/CHCl$_3$ (5:95) solution to give 7.80 g of the product. Yield: 60%. $^1$H NMR ($\delta$ (ppm),

MeOH-$d$4): 8.33 (d, $J$) 4.2 Hz, 1H, PyH), 7.67 (t, $J$) 7.5 Hz, 1H, PyH), 7.36 (d, $J$) 8.1 Hz,

1H, PyH), 7.18 (t, $J$) 6.9 Hz, 1H, PyH), 6.89 (d, $J$) 1.2 Hz, 1H, ImH), 6.71 (d, $J$) 1.2 Hz,

1H, ImH), 3.86 (s, 2H, PyCH$_2$), 3.83 (s, 2H, ImCH$_2$), 3.58 (s, 3H, NCH$_3$), 3.22 (s, 2H,

NCH$_2$), 3.99 (q, $J$) 14.4 Hz, 2H, OCH$_2$), 1.13 (t, $J$) 7.2 Hz, 3H, CH$_3$). $^{13}$C NMR ($\delta$ (ppm),

MeOH-$d$4): 172.23 (C, CO$_2$Et), 159.93 (C, Py), 149.72 (CH, Py), 146.26 (C, Im), 138.69

(CH, Py), 127.22 (CH, Py), 125.01 (CH, Py), 123.99 (CH, Im), 123.71 (CH, Im), 60.66 (C,

## Example 9

*1.1 [Re(CO)₃(N-3,5-dimethoxybenzyl-dipyridine-2-methylamine)]* **(Re-11)**



5      The [NEt₄]₂[Re(CO)₃(H₂O)₃] (0.015 g, 0.019 mmol) and 2-di(picoline)amine-*N*-3, 5-dimethoxybenzyl (KM08-121) (0.0068 g, 0.019 mmol) were placed in a 100 mL pressure tube with a stirr bar. The solids were dissolved in 5 mL of methanol. The solution was heated at 130 °C for 3 hrs. The solution was vacuumed down to residue. The residue was passed through a silca gel column using 10% methanol / methylene chloride as the solvents. The product eluted as 10   the rhenium complex (11.3 mg, 91.5 %). $^1$H NMR (CDCl₃), 300 MHz): 1.17 (s, H), 1.56 (s, 3H), 3.47 (d, H), 3.87 (s, 3H), 4.64 (m, 2H), 5.73 (d, 2H), 6.59 (t, H), 6.75 (d, H), 7.16 (t, 2H), 7.31 (m, H), 7.80 (t, 2H), 7.95 (d, 2H), 8.62 (d, 2H). LC/MS = M.W. 620. Calc. M.W. = 619.

*1.2 [Re(CO)₃(N-ethyl-ethoxy-dipyridine-2-methylamine)]* **(Re-8)**
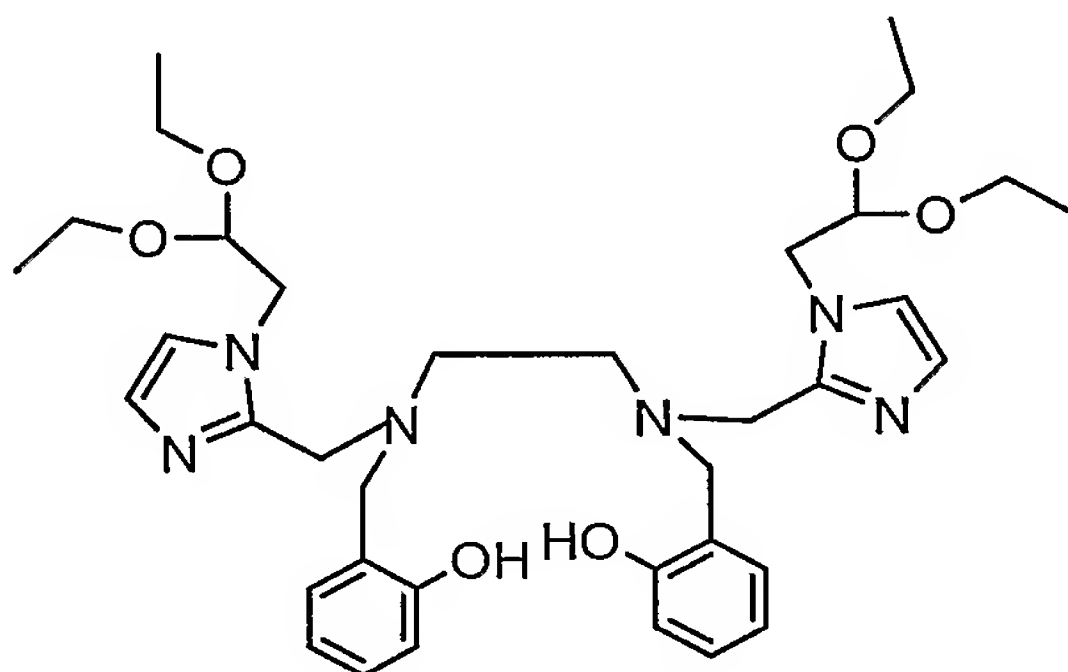


15      The [NEt₄]₂[Re(CO)₃(H₂O)₃] (0.04 g, 0.052 mmol) and 2-di(picoline)amine-*N*-ethyl ethoxy (KM08-131) (0.014 g, 0.052 mmol) were placed in a 100 mL pressure tube with a stirr bar. The solids were dissolved in 5 mL of methanol. The solution was heated at 130 °C for 2 hrs.

The solution was vacuumed down to residue. The residue was passed through a silca gel column using 10% methanol / methylene chloride as the solvents. The product eluted as the rhenium complex (8 mg, 28.6 %). $^1$H NMR (CDCl$_3$), 300 MHz): 1.25 (t, 3H), 3.72 (d, 2H), 3.97 (t, 2H), 4.05 (t, 2H), 4.55 (d, 2H), 6.10 (d, 2H), 7.18 (t, 2H), 7.80 (t, 2H), 7.95 (d, 2H), 8.62 (d, 2H).

5     LC/MS = M.W. 542.3 Calc. M.W. = 542.2.


## Example 10

*1. Synthesis of [N, N'-{N-ethyl-2-diethoxy}-2-methyl-imidazole}-N,N'-bis(2-hydroxybenzyl)-ethylenediamine]*



Placed N, N'-bis(2-hydroxybenzyl)ethylenediamine (0.1 g, 0..367 mmol) in a 100 ml round-bottom flask equipped with a stirrer under nitrogen. The solid was dissolved in 8 ml of dichloroethane, followed by addition of [N-{ethyl-2-diethoxy}-2-imidazolecarboxaldehyde] (0.165 g, 0.775 mmol) and sodium triacetoxyborohydride (0.178 g, 0.845 mmol). The solution was stirred at room temperature for 18 hours. The solution was then vacuumed down to residue. The residue was passed through a silca gel column using 0-20% methanol / methylene chloride as the solvents, yielding 0.049 g, 20.1% yield. $^1$H NMR ((CDCl$_3$), 300 MHz): 1.10 (t, 12H), 2.10 (s, 8H), 2.77 (s, 2H), 3.34 (m, 4H), 3.58 (m, 4H), 3.77 (s, 2H), 3.84 (d, 2H), 4.45 (t, 2H), 6.72 (t, 2H), 6.85 (m, 8H), 6.96 (s, 2H), 7.13 (t, 2H). ES/MS = 666-668: expected 668.


## Example 11

*1. Ethyl [(2-Pyridylmethyl)-2-(1-methylimidazolylmethyl)]aminoacetate (L$^{10}$Et).*

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To a solution of 1-methylimidazole-2-aldehyde (5 g, 45.1 mmol) in 80 mL of methanol was added slowly a solution of 2-picolylamine (4.88 g, 45.1 mmol) in 20 mL of methanol, andthe solution was stirred for 2 h. At this time, the reactants were completely consumed. To this reaction mixture was added $NaBH_4$ (1.7 g, 45.1 mmol) in portions, and the solution was stirred for another 3 h, whereupon the solution was evaporated to dryness and the residue was extracted with chloroform and concentrated. This residue was dissolved in anhydrous dimethylformamide (40 mL). Potassium carbonate (7.53 g, 45.1 mmol) and ethyl bromoacetate (6.23 g, 45.1 mmol) were added to the solution under an argon atmosphere. The resulting suspension was protected from light and allowed to stir at 30 °C, under argon, for 32 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness. The resulting red oil was purified by silica gel column chromatography using a $MeOH/CHCl_3$ (5:95) solution to give 7.80 g of the product. Yield: 60%. $^1$H NMR ($\delta$ (ppm), MeOH-$d$4): 8.33 (d, $J$) 4.2 Hz, 1H, PyH), 7.67 (t, $J$) 7.5 Hz, 1H, PyH), 7.36 (d, $J$) 8.1 Hz, 1H, PyH), 7.18 (t, $J$) 6.9 Hz, 1H, PyH), 6.89 (d, $J$) 1.2 Hz, 1H, ImH), 6.71 (d, $J$) 1.2 Hz, 1H, ImH), 3.86 (s, 2H, PyCH$_2$), 3.83 (s, 2H, ImCH$_2$), 3.58 (s, 3H, NCH$_3$), 3.22 (s, 2H, NCH$_2$), 3.99 (q, $J$) 14.4 Hz, 2H, OCH$_2$), 1.13 (t, $J$) 7.2 Hz, 3H, CH$_3$). $^{13}$C NMR ($\delta$ (ppm), MeOH-$d$4): 172.23 (C, CO$_2$Et), 159.93 (C, Py), 149.72 (CH, Py), 146.26 (C, Im), 138.69 (CH, Py), 127.22 (CH, Py), 125.01 (CH, Py), 123.99 (CH, Im), 123.71 (CH, Im), 60.66 (C, PyCH$_2$), 55.54 (C, ImCH$_2$), 51.39 (C, NCH$_3$), 33.56 (C, NCH$_2$), 61.62 (C, OCH$_2$), 14.73 (C, CH$_3$).

*2. [Re(CO)$_3$(L$^{10}$Et)]Br.*

To a stirred solution of [NEt$_4$]2[Re(CO)$_3$Br$_3$] (0.358 g, 0.465 mmol) in 40 mL of methanol was added **L$^{10}$Et** (0.134 g, 0.465 mmol) in 4 mL of methanol, and the solution was refluxed for 5 h. After cooling to room temperature, the solution was filtered and evaporated to dryness. The residue was dissolved in dichloromethane and carefully layered with hexane to give colorless crystals suitable for X-ray crystallography. Yield: 82%. Anal. Calcd (found) for $C_{18}H_{20}BrN_4O_5Re$: C, 33.86 (33.79); H, 3.16 (3.46); N, 8.77 (8.69). $^1$H NMR ($\delta$ (ppm), MeOH-$d$4): 8.80 (d, $J$) 5.4 Hz, 1H, PyH), 8.04 (t, $J$) 6.3 Hz, 1H, PyH), 7.72 (d, $J$) 7.8 Hz, 1H, PyH), 7.45 (t, $J$) 6.6 Hz, 1H, PyH), 7.14 (d, $J$) 1.8 Hz, 1H, ImH), 7.11 (d, $J$) 1.5 Hz, 1H, ImH), 5.43 (d, $J$) 16.2 Hz, 2H, PyCH$_2$), 4.85 (d, $J$) 11.7 Hz, 2H, ImCH$_2$), 4.71 (d, $J$) 4.2 Hz, 2H, NCH$_2$), 4.33 (q, $J$) 14.4 Hz, 2H, OCH$_2$), 3.60 (s, 3H, NCH$_3$), 1.36 (t, $J$) 7.2 Hz, 3H, CH$_3$). $^{13}$C NMR ($\delta$ (ppm), MeOH-$d$4): 196.91, 195.96 (*fac*-Re-CO$_3$), 170.05 (CO$_2$Et), 160.84 (C, Py), 153.92 (C,

Im), 153.34 (CH, Py), 141.72 (CH, Py), 128.75 (CH, Py), 127.17 (CH, Py), 125.66 (CH, Im),

125.43 (CH, Im), 70.69 (C, PyCH$_2$), 68.67 (C, ImCH$_2$), 63.17 (C, NCH$_2$), 59.15 (C, OCH$_2$),

34.89 (C, NCH$_3$), 14.51 (C, CH$_3$). IR (KBr, $\nu$/cm$^{-1}$): 2022, 1922, 1906 ($\nu$(fac-Re(CO)$_3$)); 1746

($\nu_{as}$(C=O)), 1208 ($\nu_{sym}$(C=O)) of the acid group.

5  *3. Ethyl [Bis{2-(1-methylimidazolylmethyl)}amino]acetate (L$^{11}$Et).*

The preparation of **L$^{11}$Et** is based on literature procedures (Oberhausen, K. J.;

Richardson, J. F.; Buchanan, R. M.; Pierce, Q. *Polyhedron* **1989**, *8*, 659; and Chen, S.;

Richardson, J. F.; Buchanan, R. M. *Inorg. Chem.* **1994**, *33*, 2376) with minor modifications.

*(a) Preparation of Bis(2-(1-methylimidazolyl)methyl)amine (L$^{11}$).* A solution of methyl-2-

10  imidazolcarboxaldehyde oxime (5 g, 40 mmol) in methanol (120 mL) was hydrogenated at

atmospheric pressure and room temperature with 10% palladium charcoal for 5 days. The

catalyst was filtered through Celite, and the filtrate was evaporated to give a white powder as **L$^{11}$**.

Yield: 78%. $^1$H NMR ($\delta$ (ppm), MeOH-$d4$): 6.85 (d, $J$) 1.2 Hz, 2H, ImH), 6.70 (d, $J$) 1.2 Hz,

2H, ImH), 3.67 (s, 4H, ImCH$_2$), 3.48 (s, 6H, NCH$_3$). $^{13}$C NMR ($\delta$ (ppm), methanol-$d4$): 147.71

15  (2C, Im), 127.25 (2CH, Im), 123.31 (2CH, Im), 45.23 (2C, ImCH$_2$), 33.37 (2C, NCH$_3$).

*(b) Preparation of L$^{11}$Et.* Potassium carbonate (2.43 g, 17.56 mmol) and ethyl

bromoacetate (1.76 g, 10.54 mmol) were added to a solution of bis(2-(1-

methylimidazolyl)methyl)amine (**L$^{11}$**) (1.8 g, 8.78 mmol) in dimethylformamide under an argon

atmosphere. The resulting suspension was sheltered from light and allowed to stir at room

20  temperature for 5 days. Water was added to the resulting mixture, and the solution was extracted

with chloroform (3 x 50 mL). After the solvent was evaporated, the resulting oil was purified by

silica gel column chromatography using a MeOH/CHCl3 (5:95) solution to give **L$^{11}$Et** as white

powder. Yield: 1.05 g (41%). $^1$H NMR ($\delta$ (ppm), MeOH-$d4$): 6.87 (d, $J$) 1.2 Hz, 2H, ImH), 6.79

(d, $J$) 1.2 Hz, 2H, ImH), 4.06 (q, $J$) 14.4 Hz, 2H, OCH$_2$), 3.75 (s, 4H, ImCH$_2$), 3.51 (s, 6H,

25  NCH$_3$), 3.35 (s, 2H, NCH$_2$CO$_2$), 1.19 (t, $J$) 7.2 Hz, 3H, CH$_3$). $^{13}$C NMR ($\delta$ (ppm), MeOH-$d4$):

172.09 (C, CO$_2$Et), 146.24 (2C, Im), 127.17 (2CH, Im), 123.79 (2CH, Im), 61.73 (C, OCH$_2$),

55.03 (2C, ImCH$_2$), 52.37 (2C, NCH$_3$), 33.24 (C, NCH$_2$), 14.67 (C, CH$_3$).

*4. [Re(CO)$_3$(L$^{11}$Et)]*

The same procedure as for [Re(CO)$_3$(**L$^{10}$Et**)] was employed. Yield: 66%. Anal. Calcd

(found) for $C_{17}H_{21}BrN_5O_5Re$: C, 31.83 (31.99); H, 3.30 (3.46); N, 10.92 (10.93). $^1H$ NMR ($\delta$ (ppm), MeOH-$d4$): 7.29 (d, $J$) 1.5 Hz, 2H, ImH), 7.17 (d, $J$) 1.5 Hz, 2H, ImH), 5.29, 4.88 (dd, $J$) 16.5 Hz, 4H, ImCH$_2$), 4.79 (s, 2H, NCH$_2$), 4.44 (q, $J$) 14.4 Hz, 2H, OCH$_2$), 3.86 (s, 6H, NCH$_3$), 1.43 (t, $J$) 7.2 Hz, 3H, CH$_3$). $^{13}C$ NMR ($\delta$ (ppm), methanol-$d4$): 197.15, 195.90 (*fac*-Re(CO)$_3$), 169.97 (C, COOEt), 152.40 (2C, Im), 128.58 (2CH, Im), 125.26 (2CH, Im), 68.77 (C, NCH$_2$), 63.12 (C, OCH$_2$), 61.18 (2C, ImCH$_2$), 35.39 (2C, NCH$_3$), 14.56 (C, CH$_3$). IR (KBr, $\nu$/cm$^-$$^1$): 2022, 1922, 1901 ($\nu$(*fac*-Re(CO)$_3$)); 1743 ($\nu_{as}$(C=O)), 1212 ($\nu_{sym}$(C=O)) of the carboxylate group.

### *Incorporation By Reference*

All of the patents and publications cited herein are hereby incorporated by reference.

### *Equivalents*

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.